the postmortem period increase only slightly with respect to liver, lung, and kidney concentrations, even for molecules with high apparent Vd such as verapamil or nortriptyline (Vd = 5.1 L/kg and 13.7 L/kg in the rat, respectively) (67). The concentration variations between the different brain areas have been the subject of only a few studies, with contradictory results. According to Moore et al. (79), ethanol concentrations in human cadavers are equal in the different sites of the gray matter and are slightly different from the white matter because of the difference in water content. These results obtained with ethanol, a small hydrophilic molecule, have been confirmed by Kalasinsky et al. (80), who measured, in 14 corpses of methamphetamine users, methamphetamine and amphetamine concentrations in 15 different brain areas and did not find statistically significant differences for these lipophilic alkaline molecules. On the contrary, Sawyer and Forney (24) found, in the rat, statistically higher free morphine concentrations in the posterior than in the anterior brain as soon as 24 h after euthanasia, as well as a subsequent significant increase with time in the posterior but not anterior brain. Though these results should be confirmed by further studies, they are in accordance with the hypothesis that the lipid solubility alone cannot explain all redistribution phenomena. On the other hand, the apparent protection of the brain from PMR is probably due to the low permeability of the blood–brain barrier with regard to the other membranes and barriers of the body. The tight junction between the endothelial cells in brain capillaries and the sleeve of glial cells around these capillaries presents a formidable obstacle to the transfer of hydrophilic compounds (except the smaller ones such as ethanol). Additionally, the thickness of membranes to be crossed and the absence of proteins in the cerebrospinal fluid (CSF) and interstitial liquid considerably slow down the transfer of large liposoluble molecules (77).

In conclusion, the PMR of a drug cannot be predicted only by its lipophilic properties and its apparent Vd. Other factors such as the absorption route, dose, or particular affinity of the drug for some tissues must be envisaged as well as the possibility of a residual metabolic activity in the first hours after death.

Metabolism

The drug-metabolizing system may persist several hours after death, inducing the breakdown of a drug and the synthesis of its metabolites. This phenomenon must be distinguished from the breakdown of drugs due to the putrefactive process. To our knowledge, all studies concerning this problem were devoted to the metabolism of cocaine. Apart from the fact that the postmortem concentrations of cocaine and its metabolites present variations according to the time of sampling and sampling sites, the hypothesis of a postmortem, residual cocaine metabolism has been raised (81). In vivo, cocaine is rapidly converted into benzoylecgonine and ecgonine methyl ester in blood and the liver. In blood, cocaine is spontaneously converted into benzoylecgonine at physiological pH, and is metabolized to ecgonine methylester by plasma cholinesterase (82,83). In the liver, cocaine is metabolized by two nonspecific hepatic esterases: one produces benzoylecgonine and the other produces ecgonine methylester (84). Cocaine is broken down gradually after death (85). According to Hearn et al. (81), this decrease in cocaine subclavian blood concentrations could not be explained by the spontaneous hydrolysis of cocaine to benzoylecgonine because hydrolysis is inhibited by the acidification of blood after death. The hypothesis of a residual esterase activity was then formulated (85). According to Isenschmid et al. (86), benzoylecgonine in postmortem blood samples arises as a result of in vivo cocaine hydrolysis and ecgonine methylester as the result of postmortem cocaine metabolism. McKinney et al. (87) confirmed this hypothesis by describing a gradual increase in postmortem ecgonine methylester femoral blood concentrations in juvenile swine treated with cocaine hydrochloride at a dose of 10 mg/kg by IV bolus, then sacrificed. On the other hand, benzoylecgonine could be produced early after death by a residual activity of hepatic cocaine-methylesterase (3). Finally, one should keep in mind that ecgonine methylester is normally found in the blood of living cocaine users (83). As far as cocaethylene is concerned, the results are more controversial. According to Logan et al. (3), the femoral and ventricular cocaethylene blood concentrations decrease gradually after death in humans, but with no consistent pattern of direction or magnitude of change, whereas in rats, Moriya and Hashimoto (88) demonstrated that cocaine is still transformed into cocaethylene in the liver of alcohol-intoxicated rats during the first hour postmortem. Even if the persistence of a residual hepatic and/or plasmatic esterase activity after death has not been clearly demonstrated, most of the authors agree with this hypothesis.

The possibility of a postmortem, residual enzymatic activity was confirmed by Moriya and Hashimoto (89) in a previous study describing the postmortem metabolism of dichlorvos, an organophosphorus pesticide, by hepatic and plasmatic esterases. Yamazaki and Wakasugi (90) studied the postmortem changes in drug-metabolizing enzymes in rat liver microsomes. The results demonstrated that the activity of the liver enzymes did not stop immediately after death, but showed a progressive decrease during the first 48 h postmortem. A residual enzymatic activity, variable with the nature of the enzymes involved, is likely during the first hours after death.

These results must be confirmed in humans, or rather in vitro using human microsomes. They underline the necessity of assaying the metabolites of the drugs studied in order to establish the parent drug/metabolite ratio for the interpretation of the results.

Elimination

As for absorption, to the best of our knowlegde no study has been dedicated to the possible persistance of an elimination process after death.

In the nephron, the processes of glomerular filtration, tubular secretion and tubular reabsorption combine to produce urine and eliminate drugs (78). Glomerular filtration, depending on the afferent blood flow, probably stops at the time of death.

Tubular secretion is an active process depending on the presence of ATP, which thus probably stops shortly after death. Conversely, tubular reabsorption is a passive process that could persist during the first postmortem hours. The acidification of plasma could modify tubular reabsorption and induce a leakage of weak acids.

Biliary excretion concerns glucuronides and polar drugs with

a molecular weight greater than 500 and less than 1000 Da (91). These highly polar molecules are concentrated in bile by active transport processes similar to those involved in the secretion of similar compounds across the renal tubular cells into urine. These active processes probably stop with the interruption of ATP synthesis. Similarly, the storage of primary bile in the gall bladder and its concentration by active water reabsorption are interrupted, as is active emptying of the gall bladder into the second duodenum.

Practical consequences in forensic toxicology

From a practical point of view, the respect of some precautionary measures can limit misinterpretations. It is very important in postmortem testing to be able to compare concentrations in several blood and tissue samples, even if reference values for drug concentrations in tissues are often missing. Blood samples must be taken at central (cardiac) and peripheral sites. In the framework of postmortem drug redistribution studies, cardiac blood samples must be taken from the right and left cardiac chambers separately, in order to determine the PMR mechanism (92). Taking into account the intensity of redistribution of certain drugs into the cardiac chambers, the estimation of the amount of drug present at the time of death from the cardiac blood concentrations must be avoided.

As for the peripheral blood sampling sites, all the authors recommend collecting blood from the femoral vein. Femoral blood appears to be the specimen of choice for postmortem toxicological analysis as it is the least subject to PMR, which, in this case, can only come from local tissues such as muscles and fat (58). Accordingly, it was found that the femoral blood concencentrations were less affected by the postmortem time delay than the concentrations in central blood (78). Femoral blood must ideally be sampled after cross-clamping the iliac vein and the inferior vena cava in order to avoid the risk of drawing blood from these vessels, but such collection is not always possible under the usual forensic autopsy conditions (20). Even if femoral blood concentrations are more representative of the antemortem blood concentrations than cardiac blood, they are frequently higher than the ante- or perimortem blood concentrations (22). More surprisingly, in a few cases, femoral blood concentrations were found to be higher than cardiac blood concentrations. This was observed in human cases where resuscitation was attempted, probably causing a shift of cardiac blood into the peripheral vessels (22,23). The same results were found in a study of the PMR of amitriptyline (93) in an experimental pig model, where the animals were sacrified using 10 mL of potassium chloride. In such a case, death is caused by ventricular fibrillation where the heart stops in diastole, inducing a shift of cardiac blood, which could explain the higher postmortem concentrations found in femoral blood. Finally, as previously explained, subclavian venous blood should not be considered as peripheral blood (23), as its drug concentration variations follow those of heart blood.

Many authors previously reported that the left lung, the left kidney and the left lobe of the liver are more prone to PMR than the right lung, the right kidney, and the right lobe of the liver because of redistribution from the gastrointestinal tract. Ideally, the right lobe of the liver, the right kidney, and the right lung should be sampled. Probably because the brain is not clearly affected by PMR, no precise recommendation concerning brain sampling is given in the literature.

Other biological matrices, less subject to PMR, have been proposed in order to avoid misinterpretations: vitreous humor, skeletal muscles, bone marrow, and CSF. Vitreous humor was the subject of many investigations and is of a great interest for forensic purposes. It contains no microorganisms or glucose, and it is also protected from putrefaction and trauma. For these reasons, it is considered a sample of choice for distinguishing exogenous from endogenous ethanol resulting from the putrefactive process (35,41,94–96). Blood ethanol concentration can be approximated from the vitreous humor concentration, taking into account that the vitreous humor/blood ethanol concentration ratio ranges from 0.9 to 1.38 (with a theoretical value of 1.27 at equilibrium) (41). Unfortunately, the concentrations of other drugs during the postmortem period cannot always be accurately estimated using vitreous humor. McKinney et al. (87) studied the PMR of cocaine in an animal model. They concluded that 8 h after death, the vitreous humor cocaine concentrations were significantly higher in all animals with respect to the concentration at the time of death and were similar to the femoral blood concentrations at the time of death. From our interpretation of the work published by Vorpahl et al. (97), there was no good quantitative correlation between vitreous humor and blood concentrations of digoxin.

The skeletal muscle has been suggested as an alternative specimen for postmortem toxicology because it is present in large amounts and is affected by decomposition later than blood or viscera (98). In addition, muscle samples can be obtained from peripheral sites, far from drug reservoirs such as the stomach, liver, and lungs (98). Langford et al. (99) evaluated the homogeneity of different drugs' concentrations (temazepam, amitriptyline, paracetamol, propoxyphene) in samples obtained from different muscles such as the diaphragm, pectoralis major, sternomastoid, deltoid, biceps, triceps, and brachioradialis. They observed a large site-to-site variablility, with drug concentrations in the diaphragm invariably higher than in the other muscles. Furthermore, the variability of these concentrations between sites, excluding the diaphragm, was more pronounced for drugs with a large Vd. The muscle is therefore more interesting for qualitative analysis than for quantitative determination of drugs (100). From our point of view, peripheral muscles (brachioradialis, sartorius) should only be sampled for qualitative screening, in cases where fluids and viscera are not available (burned cadavers, for example).

Bone marrow was also investigated as an alternative specimen (101). It is a lipid-rich matrix with a high degree of vascularity. Furthermore, its anatomical situation, encased in bones, reduces the possibility of contamination from bacteria during the putrefactive process (102). In the absence of available data concerning the correlation between bone marrow and blood concentrations, bone marrow was used to perform qualitative analysis when no other sample was available. Winek et al. (103) demonstrated that in rabbits, bone marrow could predict blood concentrations of nortriptyline up to 24 h after death. On the contrary, in pigs, Hilberg et al. (93) found no correlation between blood and bone marrow concentrations of amitriptyline

nor did they find any correlation between sternal and femoral bone marrow or between early and late sternal bone marrow. Furthermore, the dehydration of bone marrow, which takes place about 96 h after death, reduces the amounts available. Here again, bone marrow cannot be recommended as an alternative specimen to estimate the concentrations of drugs.

The CSF was also proposed as an alternative specimen, but some drugs do not diffuse into the CSF. Little is known about the evolution of drug concentrations in the CSF after death or about the possible correlation between CSF and blood drug concentrations. According to Logan and Smirnov (25), who studied the stability of morphine concentrations in CSF and their correlation with blood concentrations in 32 morphine-related death cases, CSF concentrations appeared to be stable with time. However, taking into account the large standard deviation in the mean CSF-to-femoral or iliac blood ratios, the use of CSF concentrations for the prediction of peripheral blood concentration was not advised by the authors. Furthermore, other drugs, such as amitriptyline, could be redistributed into the CSF (93).

Finally, the hematic fluid found in the declive pleural spaces is the worst biological medium for the quantitation of drugs because it is a mixture of blood and serous fluid from lungs and other thoracic organs, or even the stomach. Anyway, the lack of concentration data in the alternative specimens is another caveat of their utility in forensic investigations.

In this review, we have not considered the misinterpretations related to errors in sample preparation and preservation, such as the absence of a preservative or inappropriate storage temperatures (i.e., too high), for example.

Conclusions and Perspectives

PMR of drugs may complicate the interpretation of the results in forensic toxicology. The competing processes of diffusion from drug reservoirs, cell lysis and putrefaction, and the particular pharmacokinetic properties of certain drugs contribute to the differences in drug concentrations observed between sites and sampling intervals (3).

The most common problem is a difference in drug concentration between the different sampling sites. If these differences are moderate and especially if all these site concentrations are in the same range—therapeutic, toxic, or fatal—the interpretation may not be an issue. However, interpretation is more difficult when these concentrations are very discordant. The pharmacokinetics of the drugs concerned must be taken into account, as well as, if possible, the autopsy findings, which in many cases give useful information. The position of the corpse and regurgitation of the gastric contents into the airways or thoracic trauma may, for example, explain differences in blood concentrations from different sampling sites.

These redistribution phenomena put into perspective the reliability of the databases of therapeutic/toxic/lethal blood levels, built from published data often reported with no mention of sampling sites, postmortem delay, or autopsy conditions. This is particularly important because a large number of toxic drugs are lipophilic weak bases with a large Vd, prone to PMR.

More surprisingly, there is little information on the metabolic activity in the first hours postmortem or on the real influence of drug physicochemical properties or pharmacokinetic parameters on the redistribution phenomena. Further studies are thus warranted.

References

1. G. Skopp, R. Lutz, B. Ganßmann, R. Mattern, and R. Aderjan. Postmortem distribution pattern of morphine and morphine glucuronides in heroin overdose. *Int. J. Legal Med.* **109:** 118–124 (1996).
2. T. Nagata, K. Kimura, K. Hara, and K. Kudo. Methamphetamine and amphetamine concentrations in postmortem rabbit tissues. *Forensic Sci. Int.* **48:** 39–47 (1990).
3. B.K. Logan, D. Smirnow, and R.G. Gullberg. Lack of predictable site-dependent differences and time-dependent changes in postmortem concentrations of cocaine, benzoylecgonine and cocaethylene in humans. *J. Anal. Toxicol.* **20:** 23–31 (1997).
4. T. Hilberg, A. Bugge, K.M. Beylich, J. Ingum, A. Bjørneboe, and J. Mørland. An animal model of postmortem amitriptyline redistribution. *J. Forensic Sci.* **38:** 81–90 (1993).
5. D.J. Pounder and D.R.W. Smith. Postmortem diffusion of alcohol from the stomach. *Am. J. Forensic Med. Pathol.* **16:** 89–96 (1995).
6. C. Fuke, C.L. Berry, and D.J. Pounder. Postmortem diffusion of ingested and aspirated paint thinner. *Forensic Sci. Int.* **78:** 199–207 (1996).
7. R. Pohland and N.R. Bernhard. Postmortem serum and tissue redistribution of fluoxetine and norfluoxetine in dogs following oral administration of fluoxetine hydrochloride (Prozac®). *J. Forensic Sci.* **42:** 812–816 (1997).
8. D.J. Pounder, C. Fuke, D.E. Cox, D. Smith, and N. Kuroda. Postmortem diffusion of drugs from gastric residue. *Am. J. Forensic Med. Pathol.* **17:** 1–7 (1996).
9. J.V. Marraccini, T. Carroll, S. Grant, S. Halleran, and J.A. Benz. Differences between multisite postmortem ethanol concentrations as related to agonal events. *J. Forensic Sci.* **35:** 1360–1366 (1990).
10. D.J. Pounder. Postmortem diffusion of tracheal lidocaine into heart blood following intubation for cardiopulmonary resuscitation. *J. Forensic Sci.* **42:** 965–966 (1997).
11. D.J. Pounder and K. Yonemitsu. Postmortem absorption of drugs and ethanol from aspirated vomitus—an experimental model. *Forensic Sci. Int.* **51:** 189–195 (1991).
12. A.C. MacIntyre and D.J. Cutler. The potential role of lysosomes in tissue distribution of weak bases. *Biopharm. Drug. Dispos.* **9:** 513–526 (1988).
13. T. Miyazaki, T. Kojima, M. Yashiki, H. Wakamoto, Y. Iwasaki, and T. Taniguchi. Site dependence of methamphetamine concentrations in blood samples collected from cadavers of people who had been methamphetamine abusers. *Am. J. Forensic Med. Pathol.* **14:** 121–124 (1993).
14. W.A. Daniel, M.H. Bickel, and U.E. Honegger. The contribution of lysosomal trapping in the uptake of desipramine and chloroquine by different tissues. *Pharmacol. Toxicol.* **77:** 402–406 (1995).
15. T. Hilberg, J. Mørland, and A. Bjørneboe. Postmortem release of amitriptyline from the lungs; a mechanism of postmortem drug redistribution. *Forensic Sci. Int.* **64:** 47–55 (1994).
16. F. Moriya and Y. Hashimoto. Postmortem diffusion of tracheal lidocaine into heart blood following intubation for cardiopulmonary resuscitation. *J. Forensic Sci.* **42:** 296–299 (1997).
17. D.J. Pounder, V. Owen, and C. Quigley. Postmortem changes in blood amitriptyline concentration. *Am. J. Forensic Med. Pathol.* **15:** 224–230 (1994).

9-14

Case 2:08-md-01968 Document 580-27 Filed 09/08/11 Page 4 of 64 PageID #: 24479

18. F. Moriya and Y. Hashimoto. Redistribution of basic drugs into cardiac blood from surrounding tissues during early-stages postmortem. *J. Forensic Sci.* **44:** 10–16 (1999).
19. T. Hilberg, A. Bugge, K.M. Beylich, J. Mørland, and A. Bjømeboe. Diffusion as a mechanism of postmortem drug redistribution: an experimental study in rats. *Int. J. Legal Med.* **105:** 87–91 (1992).
20. D.J. Pounder. The nightmare of postmortem drug changes. In *Legal Medicine 1993*, C.H. Wecht, Ed. Butterworth Legal Publishers, Salem, NH, 1994, pp 163–193.
21. D.J. Pounder and J.I. Davies. Zopiclone poisoning: tissue distribution and potential for postmortem diffusion. *Forensic Sci. Int.* **65:** 177–183 (1994).
22. T. Hilberg, S. Rogde, and J. Mørland. Postmortem drug redistribution—human cases related to results in experimental animals. *J. Forensic Sci.* **44:** 3–9 (1999).
23. R.W. Prouty and W.H. Anderson. The forensic science implications of site and temporal influences on postmortem blood-drug concentrations. *J. Forensic Sci.* **35:** 243–270 (1990).
24. W.R. Sawyer and R.B. Forney. Postmortem disposition of morphine in rats. *Forensic Sci. Int.* **38:** 259–273 (1988).
25. B.K. Logan and D. Smirnow. Postmortem diffusion and redistribution of morphine in man. *J. Forensic Sci.* **41:** 37–46 (1996).
26. F.E. Barnhart, J.R. Fogacci, and D.W. Reed. Methamphetamine—a study of postmortem redistribution. *J. Anal. Toxicol.* **23:** 69–70 (1999).
27. G.R. Jones and D.J. Pounder. Site dependence of drug concentrations in postmortem blood—a case study. *J. Anal. Toxicol.* **11:** 186–190 (1987).
28. R.S. Cotran, V. Kumar, and S.L. Robbins. Cellular injury and adaptation. In *Robbin's Pathologic Basis of Disease*, 4th ed. W.B. Saunders, Philadelphia, PA, 1989, pp 1–38.
29. A.M. Langford and D.J. Pounder. Possible markers for postmortem drug redistribution. *J. Forensic Sci.* **42:** 88–92 (1997).
30. G. Skopp, R. Lutz, L. Pötsch, B. Ganßmann, K. Klinder, A. Schmidt, R. Aderjan, and R. Mattern. An in vitro experiment for postmortem vascular permeation. The passage of morphine and morphine glucuronides accross a vascular wall. *J. Forensic Sci.* **42:** 486–491 (1997).
31. H. Thomsen, H.J. Kaatsch, and B. Krisch. How and why does the platelet count in postmortem blood change the early postmortem interval? *J. Forensic Sci.* **101:** 185–194 (1999)
32. M. Durigon. *Pratique Médico-légale*. Masson, Paris, France, 1999, pp 39–49.
33. M. Fallani. Contributo allo studio della circolazione ematica post-mortale. *Minerva Medicoleg.* **81:** 108–115 (1961).
34. M. Gomez-Zapata, M. Alcaraz, and A. Luna. Studies on postmortem circulation of the blood. *Z. Rechtsmed.* **103:** 27–32 (1989).
35. C.L. O'Neal and A. Poklis. Postmortem production of ethanol and factors that influence interpretation: a critical review. *Am. J. Forensic Med. Pathol.* **17:** 8–20 (1996).
36. J.E.L. Corry. Possible sources of ethanol ante- and post-mortem: its relationship to the biochemistry and microbiology of decomposition. *J. Appl. Bacteriol.* **44:** 1–56 (1978).
37. A. Bouillerot and C. Laviano-Rousselin. Dosage d'éthanol: les erreurs pré-analytiques. Alcools et Glycols. *Journée thématique de la Société Française de Toxicologie Analytique*. Paris, France, 8 décembre 1999.
38. D.M. Kupfer, A.K. Chaturvedi, D.V. Canfield, and B.A Roe. PCR-based identification of postmortem microbial contaminants—a preliminary study. *J. Forensic Sci.* **44:** 592–596 (1999).
39. W.D. Alexander. Postmortem urinary alcohol is unreliable in diabetes. *Br. Med. J.* **317:** 206 (1998).
40. G.L. Davis, R.L. Leffert, and N.W. Rantanen. Putrefactive ethanol sources in postmortem tissues of conventional and germ-free mice. *Arch. Pathol.* **94:** 71–74 (1972).
41. E.J. Briglia, J.H. Bidanset, and L.A. Dal Cortivo. The distribution of ethanol in postmortem blood specimens. *J. Forensic Sci.* **37:** 991–998 (1992).
42. A.W. Jones, R. Andersson, J. Sakshaug, and J. Mørland. Possible formation of ethanol in postmortem blood specimen after antemortem treatment with mannitol. *J. Anal. Toxicol.* **15:** 157–158 (1991).
43. M. Deveaux. Alcool éthylique. In *Toxicologie et Pharmacologie Médicolégales*, P. Kintz, Ed. Elsevier, Paris, France, 1998, pp 111–126.
44. M.G. Gilliland and R.O. Bost. Alcohol in decomposed bodies: postmortem synthesis and distribution. *J. Forensic Sci.* **38:** 1266–1274 (1993).
45. W. Grellner and R. Iffland. Assessment of postmortem blood alcohol concentrations by ethanol levels measured in fluids from putrefactive blisters. *Forensic Sci. Int.* **90:** 57–63 (1997).
46. C. Laviano. Production et consommation d'éthanol post-mortem dans deux liquides biologiques. *Ann. Biol. Clin.* **56:** 96–99 (1998).
47. T. Takayasu, T. Ohshima, N. Tanaka, H. Maeda, T. Kondo, J. Nishigami, and T. Nagano. Postmortem degradation of administered ethanol-d6 and production of endogenous ethanol: experimental studies using rats and rabbits. *Forensic Sci. Int.* **76:** 129–140 (1995).
48. M.D. Robertson and O.H. Drummer. Postmortem distribution and redistribution of nitrobenzodiazepines in man. *J. Forensic Sci.* **43:** 9–13 (1998).
49. B. Ballantyne, J.E. Bright, and P. Williams. The post-mortem rate of transformation of cyanide. *Forensic Sci.* **3:** 71–76 (1974).
50. W.L. Chiou. The phenomenon and rationale of marked dependence of drug concentration on blood sampling site. Implications in pharmacokinetics, pharmacodynamics, toxicology and therapeutics (Part I). *Clin. Pharmacokinet.* **17:** 175–199 (1989).
51. A.A. Elsirafy, A.A. Ghanem, A.E. Eid, and S.A. Eldakroory. Chronological study of diazinon in putrefied viscera of rats using GC/MS, GC/EC, and TLC. *Forensic Sci. Int.* **109:** 147–157 (2000).
52. J.P. Tillement and E. Lindenlaub. *Protein Binding and Drug Transport*. F.K. Schattauer Verlag, Stuttgart, Germany, 1986.
53. A.S. Konikova, A.A. Vinarskaya, V.I. Nikulin, A.V. Pogossova, and L.M. Petukhova. Protein degradation to low-molecular compounds after death and during reanimation. *Virchows Arch. B. Cell. Pathol.* **18:** 347–355 (1975).
54. W. Bonte, J. Bleifuss, and J. Volck. Experimental investigations in post-mortem protein degradation. *Forensic Sci.* **7:** 9–22 (1976).
55. M. Oehmichen and M. Gencic. Postmortal diffusion of plasma albumin in rat brain. *Z. Rechtsmed.* **84:** 113–123 (1980).
56. T. Tomson, A.C. Sköld, P. Holmgen, L. Nilsson, and B. Danielsson. Postmortem changes in blood concentrations of phenytoin and carbomazepine: an experimental study. *Ther. Drug Monit.* **20:** 309–312 (1998).
57. G. Skopp, L. Pötsch, B. Ganßmann, R. Aderjan, and R. Mattern. A preliminary study on the distribution of morphine and its glucuronides in the subcompartiments of blood. *J. Anal. Toxicol.* **22:** 261–264 (1998).
58. D.S. Cook, R.A. Braithwaite, and K.A. Hale. Estimating antemortem drug concentrations from postmortem blood samples: the influence of postmortem redistribution. *J. Clin. Pathol.* **53:** 282–285 (2000).
59. M.J. Ellenhorn and D.G. Barceloux. *Medical Toxicology, Diagnosis and Treatment of Human Poisoning*. Elsevier, New York, NY, 1988, pp 104–130.
60. A. Martin and D.J. Pounder. Post-mortem toxico-kinetics of trazodone. *Forensic Sci. Int.* **56:** 201–207 (1992).
61. D.J. Pounder, A.K. Hartley, and P.J. Watmough. Postmortem redistribution and degradation of dothiepin. *Am. J. Forensic Med. Pathol.* **15:** 231–235 (1994).
62. T. Keller, A. Schneider, and E. Tutsch-Bauer. Fatal intoxication due to dothiepin. *Forensic Sci. Int.* **109:** 159–166 (2000).
63. P.D. Jaffe, H.P. Batziris, P. van der Hoeven, D. DeSilva, and I.M. McIntyre. A study involving venlafaxine overdoses: comparison of fatal and therapeutic concentrations in postmortem specimens. *J. Forensic Sci.* **44:** 193–196 (1999).
64. B. Levine, S.C. Wu, A. Dixon, and J.E. Smialek. Site dependence

PLAINTIFFS' EXHIBITS 012597

9-15

Case 2:08-md-01968 Document 580-27 Filed 09/08/11 Page 5 of 64 PageID #: 24480

of postmortem blood methadone concentrations. *Am. J. Forensic Med. Pathol.* **16:** 97–100 (1995).
65. C.M. Milroy and A.R. Forrest. Methadone deaths: a toxicological analysis. *J. Clin. Pathol.* **53:** 277–281 (2000).
66. J.J. O'Sullivan, P.T. McCarthy, and C. Wren. Differences in amiodarone, digoxin, flecaidine and sotalol concentrations between antemortem serum and femoral postmortem blood. *Hum. Exp. Toxicol.* **14:** 605–608 (1995).
67. T. Hilberg, A. Ripel, L. Slørdal, A. Bjørneboe, and J. Mørland. The extent of postmortem drug redistribution in a rat model. *J. Forensic Sci.* **44:** 956–962 (1999).
68. J. Gerostamoulos and O.H. Drummer. Postmortem redistribution of morphine and its metabolites. *J. Forensic Sci.* **45:** 843–845 (2000).
69. S. Felby, H. Christensen, and A. Lund. Morphine concentrations in blood and organs in cases of fatal poisoning. *J. Forensic Sci.* **3:** 77–81 (1974).
70. S.C. Chan, E.M. Chan, and H.A. Kaliciak. Distribution of morphine in body fluids and tissues in fatal overdose. *J. Forensic Sci.* **31:** 1487–1491 (1986).
71. P. Carrupt, B. Testa, A. Bechalany, N. El Tayar, P. Descas, and D. Perrissoud. Morpine-6-glucuronide and morphine-3-glucuronide as molecular chameleons with unexpected lipophilicity. *J. Med. Chem.* **34:** 1272–1275 (1991).
72. A.C. Moffat, J.V. Jackson, M.S. Moss, and B. Widdop. *Clarke's Isolation and Identification of Drugs*, 2nd ed. Pharmaceutical Press, London, U.K., 1986, pp 849–850.
73. H.F. Gomez, P. McKinney, S. Phillips, D.V. Roberts, J. Brent, and W.A. Watson. Postmortem acetaminophen pharmacokinetics: an experimental study of site and time dependent concentration changes. *J. Forensic Sci.* **40:** 980–982 (1995).
74. D.J. Gee. The morbid anatomist's role in drug detection. In *Ciba Foundation Symposium No 26. The Poisoned Patient: the Role of the Laboratory*. Associated Scientific, Amsterdam, the Netherlands, 1974, pp 239–251.
75. C.J. Timmer, J.M. Ad Sitsen, and L.P. Delbressine. Clinical pharmacokinetics of mirtazapine. *Drug Dispos.* **38:** 461–474 (2000).
76. D.T. Anderson, K.L. Fritz, and J.J. Muto. Distribution of mirtazapine (Remeron®) in thirteen postmortem cases. *J. Anal. Toxicol.* **23:** 544–548 (1999).
77. K.A. Moore, B. Levine, M.L. Smith, S. Saki, J. Schames, and J.E. Smialek. Tissue distribution of mirtazapine (Remeron®) in postmortem cases. *J. Anal. Toxicol.* **23:** 541–543 (1999).
78. P. Marquet and G. Lachâtre. Devenir des xénobiotiques dans l'organisme. In *Toxicologie et Pharmacologie Médicolégales*, P. Kintz, Ed. Elsevier, Paris, France, 1998, pp 27–66.
79. K.A. Moore, G.W. Kunsman, B.S. Levine, M.M. Herman, J. Cervenak, and T.M. Hyde. A comparison of ethanol concentrations in the occipital lobe and cerebellum. *Forensic Sci. Int.* **86:** 127–134 (1997).
80. K.S. Kalasinsky, T.Z. Bosy, G.A. Schmunk, G. Reider, R.M. Anthony, Y. Furukawa, M. Guttman, and S.J. Kish. Regional distribution of methamphetamine in autopsied brain of chronic human methamphetamine users. *Forensic Sci. Int.* **116:** 163–169 (2001).
81. W.L. Hearn, E.E. Keran, H. Wei, and G. Hime. Site-dependent postmortem changes in blood cocaine concentrations. *J. Forensic Sci.* **36:** 673–684 (1991).
82. D. Stewart, T. Inaba, M. Lucassen, and M. Kalow. Cocaine metabolism: cocaine and norcocaine hydrolysis by liver and serum esterases. *Clin. Pharm. Ther.* **25:** 464–468 (1979).
83. D. Stewart, T. Inaba, B. Tang, and M. Kalow. Hydrolysis of cocaine in human plasma by cholinesterase. *Life Sci.* **20:** 1557–1564 (1977).
84. R.A. Dean, J. Zhang, M.R. Brzezinski, and W.F. Bosron. Tissue distribution of cocaine methyl esterase and ethyl transferase activities: correlation with carboxylesterase protein. *J. Pharmacol. Exp. Ther.* **275:** 965–971 (1995).
85. F. Moriya and Y. Hashimoto. The effect of postmortem interval on the concentrations of cocaine and cocaethylene in blood and tissues: an experiment using rats. *J. Forensic Sci.* **41:** 129–133 (1996).
86. D.S. Isenschmid, B.S. Levine, and Y.H. Caplan. The role of ecgonine methyl ester in the interpretation of cocaine concentrations in postmortem blood. *J. Anal. Toxicol.* **16:** 319–324 (1992).
87. P.E. McKinney, S. Phillips, H.F. Gomez, J. Brent, M. MacIntyre, and W.A. Watson. Vitreous humor cocaine and metabolite concentrations: do postmortem specimens reflect blood levels at the time of death? *J. Forensic Sci.* **40:** 102–107 (1995).
88. F. Moriya and Y. Hashimoto. Postmortem stability of cocaine and cocaethylene in blood and tissues of humans and rabbits. *J. Forensic Sci.* **41:** 612–616 (1996).
89. F. Moriya and Y. Hashimoto. Comparative studies on tissue distributions of organophosphorus, carbamate and organochlorine pesticides in decedents intoxicated with these chemicals. *J. Forensic Sci.* **44:** 1131–1135 (1999).
90. M. Yamazaki and C. Wakasugi. Postmortem changes in drug-metabolizing enzymes of rat liver microsome. *Forensic Sci. Int.* **67:** 155–168 (1994).
91. P. Lechat. Elimination des médicaments. In *Pharmacologie Médicale*, 5th ed. P. Lechat, F. Calvo, P. de Crémoux, J.P. Giroud, G. Lagier, P. Lechat, B. Rouveix, and S. Weber, Eds. Masson, Paris, France, 1990, pp 86–91.
92. F. Moriya and Y. Hashimoto. Redistribution of methamphetamine in the early postmortem period. *J. Anal. Toxicol.* **24:** 153–155 (2000).
93. T. Hilberg, A. Ripel, A.J. Smith, L. Slørdal, J. Mørland, and A. Bjørneboe. Postmortem amitriptyline pharmacokinetics in pigs after oral and intravenous routes of administration. *J. Forensic Sci.* **43:** 380–387 (1998).
94. D.J. Pounder, E. Adams, C. Fuke, and A. Langford. Site to site variability of postmortem drug concentrations in liver and lung. *J. Forensic Sci.* **41:** 927–932 (1996).
95. M. Deveaux and D. Gosset. Le vitré: un milieu inconnu en toxicologie médico-légale. *Toxicorama* **8:** 15–20 (1996).
96. I.V. Lima and A.F. Midio. Origin of blood ethanol in decomposed bodies. *Forensic Sci. Int.* **106:** 157–162 (1999).
97. T.E. Vorpahl and J.I. Coe. Correlation of antemortem and postmortem digoxin levels. *J. Forensic Sci.* **23:** 329–234 (1978).
98. K.R. Williams and D.J. Pounder. Site-to-site variability of drug concentrations in skeletal muscle. *Am. J. Forensic Med. Pathol.* **18:** 246–250 (1997).
99. A.M. Langford, K.K. Taylor, and D.J. Pounder. Drug concentration in selected skeletal muscles. *J. Forensic Sci.* **43:** 22–27 (1998).
100. J.C. Garriott. Skeletal muscle as an alternative specimen for alcohol and drug analysis. *J. Forensic Sci.* **36:** 60–69 (1991).
101. T.T. Noguchi. Drug analysis in skeletonizing remains. *J. Forensic Sci.* **23:** 490–492 (1978).
102. C.L. Winek, S.E. Westwood, and W.W. Wahba. Plasma versus bone marrow desipramine: a comparative study. *Forensic Sci. Int.* **48:** 49–57 (1990).
103. C.L. Winek, E.M. Morris, and W.W. Wahba. The use of bone marrow in the study of postmortem redistribution of nortriptyline. *J. Anal. Toxicol.* **17:** 93–98 (1993).

Manuscript received November 25, 2002;
revision received May 22, 2003.

PLAINTIFFS' EXHIBITS 012598

9-16

Gideon Koren,[1] *M.D. and Stuart M. MacLeod,*[2] *M.D., Ph.D.*

Postmortem Redistribution of Digoxin in Rats

REFERENCE: Koren, G. and MacLeod, S. M., **"Postmortem Redistribution of Digoxin in Rats,"** *Journal of Forensic Sciences*, JFSCA, Vol. 30, No. 1, Jan. 1985, pp. 92–96.

ABSTRACT: Adult male Wistar rats were treated with either 0.1 or 3 mg/kg body weight · day of digoxin for five days, then killed and stored at 4°C for 12 h in an attempt to mimic the normal preautopsy procedures in our hospital. In rats treated with 0.1 mg/kg body weight · day, the antemortem serum digoxin concentrations (SDC) were 1.1 ± 0.4 ng/mL while the 12-h postmortem concentration was markedly increased (16.3 ± 5.9 ng/mL) ($P < 0.01$). In rats treated with 3 mg/kg body weight · day, SDC was not changed significantly (11.2 ± 4.8 ng/mL antemortem and 13.3 ± 6 ng/mL postmortem). Postmortem redistribution of digoxin was assessed by injection of ^{125}I-labelled digoxin with or without pretreatment with the unlabelled drug. The results indicate that after death passive redistribution of digoxin may take place. When the SDC are within the therapeutic or low toxic range, digoxin may reenter the blood. High antemortem serum concentrations of digoxin may prevent such passive redistribution. Therefore, antemortem digoxin intoxication cannot be reliably inferred on the basis of high postmortem levels of the drug. Digoxin intoxication can be ruled out when postmortem SDC remain within the therapeutic range. The above changes cast doubt on some of the forensic and cardiologic literature, which has in the past been based on incorrect assumptions concerning postmortem behavior of digoxin.

KEYWORDS: pathology and biology, digoxin, blood, postmortem examinations, pharmacokinetics, redistribution

Digitalis intoxication is a serious clinical emergency that, in adults, has been reported to be associated with digoxin serum concentrations higher than 2.5 ng/mL [*1*]. Since the drug is frequently administered to critically ill patients, the possibility of digitalis intoxication must be considered in every unexplained death of a digitalized patient [*2*]. Recently, several studies have reported postmortem serum digoxin concentrations significantly higher than those normally measured during life [*3–5*]. Holt [*6*] and Doherty [*7*] have suggested that after death a new equilibrium between the blood and tissues is established, resulting in a higher digoxin concentration in the blood. However, no controlled experiment has been reported to prove this assumption.

The phenomenon does create difficulties in interpretation of postmortem serum digoxin levels in cases where antemortem serum levels are not available. Moreover, studies in which postmortem tissue versus plasma concentrations of digoxin have been assessed are further confounded since it is possible that these values may not reflect the normal distribution of the drug in life, but rather a new and radically altered distribution [*5,8–10*]. There are no studies of changing digoxin distribution in the terminal stages of either acute or chronic cardiac failure. The available data imply that substantial shifts in distribution may occur.

It was the aim of our studies to describe any discrepancies that may exist between antemortem and postmortem digoxin levels in the blood and in various tissues using both thera-

Received for publication 15 March 1984; revised manuscript received 28 April 1984; accepted for publication 21 May 1984.

[1] Fellow, The Hospital for Sick Children Foundation, Toronto.
[2] Chief, Division of Clinical Pharmacology, The Hospital for Sick Children, Toronto.

peutic a
studied

Material

Antemor

Sixtee
weight (
chosen b
[*11*] and
were kill
obtained
12 h, in
storage
the hear
gland N

Postmor

Five a
England
killed b
were ren
counter
blood).
at 4°C f
reassess
In the
results.
In a f
treated
were inj
belled d
liver, ar
was mea
of blood
erator a
to the s
Resul
simultai

Results

The n
dose of
mL), wh
ng/mL)
In th
mortem
In this
nificant

peutic and toxic digoxin levels in a rat model. In the second stage of this experiment we studied possible postmortem redistribution of the drug using radiolabelled digoxin.

Materials and Methods

Antemortem and Postmortem Digoxin Serum Levels

Sixteen adult male Wistar rats were treated with either 0.1 (eight rats) or 3 mg/kg body weight (eight rats) of intramuscular digoxin per day for five days. These dose regimens were chosen because of the high LD_{50} of digoxin in the rat, which exceeds by far the human values [11] and the rapid elimination rate of the cardiac glycoside in rodents. On the sixth day they were killed by cervical dislocation and serum samples for measurement of digoxin levels were obtained immediately from the heart. Carcasses were then stored in a refrigerator at 4°C for 12 h, in an attempt to mimic the normal preautopsy procedures in our hospital. After this storage period samples for measurement of digoxin concentrations were again obtained from the heart. Digoxin serum levels were assessed by the routine radioimmunoassay (New England Nuclear Ltd.).

Postmortem Redistribution of Digoxin

Five adult male Wistar rats were injected intramuscularly with ^{125}I-labelled digoxin (New England Nuclear) 0.015 μCi with specific activity of 2000 dpm/pg. Two hours later they were killed by cervical dislocation and samples of cardiac muscle, diaphragm, liver, and kidney were removed. Renal cortical, liver, heart, and diaphragm radioactivity was measured in a γ counter (dpm per gram of wet tissue) and compared to the blood radioactivity (per gram of blood). The carcasses of these five rats were then stored as described above in a refrigerator at 4°C for 12 h, following which various tissue samples were again removed, radioactivity reassessed and compared to blood radioactivity.

In the above studies comparisons were made by the two-tailed student's *t* test for unpaired results.

In a further study of postmortem digoxin redistribution five adult male Wistar rats were treated for five days with unlabelled digoxin 1 mg/kg body weight. On the sixth day they were injected with ^{125}I-labelled digoxin 0.015 μCi, 2 h after the daily injection of the unlabelled drug. Two hours later they were killed and samples of cardiac muscle, diaphragm, liver, and kidney were removed. Renal cortical, liver, heart, and diaphragm radioactivity was measured (dpm per gram of wet tissue) and compared to blood radioactivity (per gram of blood). As in earlier experiments, the carcasses were subsequently maintained in a refrigerator at 4°C for 12 h, following which the radioactivity of the various tissues was compared to the serum reactivity.

Results are expressed throughout the text as mean ± standard deviation. Results from simultaneous studies were compared by the two-tailed student's *t* test for paired results.

Results

The mean digoxin concentration of serum obtained from heart of rats treated with digoxin dose of 0.1 mg/kg body weight was within the therapeutic range for humans (1.1 ± 0.4 ng/mL), while the mean 12-h postmortem concentration was markedly increased (16.3 ± 5.9 ng/mL) ($P < 0.01$).

In the group of rats treated with a high digoxin dosage (3 mg/kg body weight) the antemortem level of serum digoxin was within the toxic range for humans (11.2 ± 4.8 ng/mL). In this group the mean serum concentration although slightly increased did not change significantly 12 h after death (13.3 ± 6 ng/mL).

9-18

Tissue: Plasma Distribution

Animals Injected with Radiolabelled Digoxin—The tissue: blood distribution ratio of ^{125}I digoxin is shown in Table 1. The antemortem data indicate high tissue: blood ratio of digoxin in the kidney, liver, diaphragm, and cardiac muscle.

In the 12-h postmortem specimens, the concentration of the labelled digoxin in the blood was much higher than found in the antemortem samples (960 and 155 cpm/g, respectively, $P < 0.001$). Primarily because of this increase in blood digoxin concentration, tissue: blood ratios for labelled digoxin significantly decreased to approach unity in all tissues examined.

Previously Digitalized Animals Injected with Radiolabelled Digoxin—The tissue: blood distribution ratio of ^{125}I digoxin in animals given radioactive digoxin after earlier digitalization is shown in Table 2. The antemortem data demonstrate low tissue: blood ratios in the various tissues studied. These ratios are significantly lower than those observed in undigitalized rats receiving a single injection with radiolabelled digoxin ($P < 0.05$). Twelve hours later the tissue: blood ratio of labelled digoxin was found to be unchanged in the digitalized rats in all tissues tested.

Discussion

In common with earlier reported human studies [3–5], the first part of our experiment indicates that in the rat low antemortem serum levels during life tend to increase significantly after death. On the other hand, this phenomenon was not observed following exposure of test animals to higher digoxin dosage. In that situation the postmortem levels were similar to the higher antemortem concentrations. The combination of these two observations leads to the suggestion that passive redistribution of digoxin may occur after death. During life it appears that most of the drug is actively accumulated by cardiac and skeletal muscle as well as by kidney and liver [12]. The tissue: serum digoxin ratio during life is high above unity for these tissues, accounting for the large distribution volume of the drug [12]. Spiehler has found high concentration of digoxin in the brain of toxic cases and not of therapeutic

TABLE 1—*Antemortem and 12-h postmortem tissue: blood distribution ratio of ^{125}I digoxin in undigitalized rats injected with the radiolabelled digoxin 2 h before being killed.*

Ratio	Antemortem	12-h Postmortem	Significance of Change
Kidney: blood	7.9 ± 5.4	1.1 ± 0.5	$P < 0.05$
Liver: blood	8.8 ± 2.3	1.2 ± 0.3	$P < 0.01$
Cardiac: blood	10.6 ± 6.6	0.9 ± 0.2	$P < 0.05$
Diaphragm: blood	6.1 ± 1.3	0.8 ± 0.2	$P < 0.05$

TABLE 2—*Antemortem and 12-h postmortem tissue: blood distribution ratio of ^{125}I digoxin in rats exposed for five days to toxic doses of the drug.*

Ratio	Antemortem	12-h Postmortem	Significance of Change
Kidney: blood	2.4 ± 0.2	2.3 ± 0.3	N.S.[a]
Liver: blood	0.9 ± 0.2	0.9 ± 0.2	N.S.
Cardiac: blood	0.9 ± 0.2	0.9 ± 0.3	N.S.
Diaphragm: blood	1.0 ± 0.2	1.6 ± 0.6	N.S.

[a] N.S. = No significance.

cases, thus suggesting that digoxin content of the medulla may be useful in confirmation of antemortem blood digoxin concentrations [13]. After death, it appears that cessation of the active modulating accumulation process takes place, and, as a result, digoxin is redistributed passively from tissues containing digoxin in high concentration into areas of lower concentrations such as the blood. On the other hand, when serum concentrations of digoxin are extremely high because of acute intoxication the lack of a gradient may block redistribution.

To study empirically this hypothesis, we monitored postmortem digoxin redistribution using digoxin labelled with ^{125}I. We measured the tissue:blood ratios for various tissues at the time of death and 12 h later. Our results indicate that in undigitalized rats given an acute dose of digoxin, digoxin accumulates during life in the various tissues in concentrations much higher than the serum concentrations. These results are consistent with DiGregorio's observations on the tissue distribution of digoxin in the rat [12], as well as with human studies [5,8–10].

The tissue:blood concentration ratio for digoxin 12 h after death approaches unity, indicating that in the various tissues equilibrium of digoxin concentrations with blood concentrations has been achieved. This indicates that after death the drug tends to leave the cells and to enter the extracellular as well as the intravascular compartment.

Conversely, redistribution of digoxin was inhibited in animals previously exposed to pretreatment with toxic doses of the drug in nonlabelled form. The radiolabelled digoxin given after such pretreatment did not enter tissues in large quantities in these animals probably because of earlier saturation of digoxin binding sites by the excessive amounts of unlabelled digoxin. During the 12 h after death a redistribution of digoxin did not take place as a result of the relative balance between the organ:blood distribution already established in the digitalized animals.

Our findings have several implications for the interpretation of postmortem digoxin levels in serum as well as in various tissue.

1. After death, passive redistribution of digoxin may take place. When the serum concentrations are within the therapeutic or low toxic range it appears likely that digoxin will reenter the blood. High antemortem serum concentrations of digoxin may prevent such a passive redistribution.

2. Antemortem digoxin intoxication cannot be reliably inferred on the basis of high postmortem levels of the drug alone.

3. Digoxin intoxication can be ruled out when postmortem serum concentrations remain within the therapeutic range.

4. Since the redistribution of digoxin depends upon the time after death, and probably on other, as yet unknown factors, any extrapolation from postmortem data to the distribution of the drug in life may be tenuous. The changes reported above cast doubt on some of the cardiologic literature [10–11,14], which have reported postmortem tissue digoxin concentrations as if these values accurately represent the antemortem distribution of the drug.

There is a pressing need for better postmortem human studies of digoxin distribution for purposes of both medicolegal and clinical understanding.

Acknowledgment

The authors wish to thank Ms. Linda Citren for excellent secretarial help and Dr. S. P. Spielberg for thoughtful advice.

References

[1] Smith, T. W. and Haber, E., "Digoxin Intoxication: The Relationship of Clinical Presentation to Serum Digoxin Concentration," *Journal of Clinical Investigation*, Vol. 49, 1970, pp. 2377–2386.

[2] Beller, G. A., Smith, T. W., Abelman, W. H., Huber, E., and Hood, W. B., "Digitalis Intoxica-

distribution ratio of ^{125}I
tissue:blood ratio of di-

lled digoxin in the blood
.5 cpm/g, respectively, P
centration, tissue:blood
in all tissues examined.
oxin—The tissue:blood
n after earlier digitaliza-
ssue:blood ratios in the
se observed in undigita-
< 0.05). Twelve hours
anged in the digitalized

part of our experiment
tend to increase signifi-
bserved following expo-
postmortem levels were
f these two observations
cur after death. During
c and skeletal muscle as
uring life is high above
f the drug [12]. Spiehler
and not of therapeutic

tribution ratio
ed digoxin 2 h

ignificance of Change
$P < 0.05$
$P < 0.01$
$P < 0.05$
$P < 0.05$

tribution ratio
the drug.

ignificance of Change
N.S.[a]
N.S.
N.S.
N.S.

9-20

tion: A Prospective Clinical Study with Serum Level Correlation," *New England Journal of Medicine*, Vol. 284, 1971, pp. 980–997.

[3] Vorpahl, T. E. and Coe, J. I., "Correlation of Antemortem and Postmortem Digoxin Levels," *Journal of Forensic Sciences*, Vol. 23, No. 2, April 1978, pp. 329–334.

[4] Karjalaimen, J., Ojala, K., and Reissel, P., "Tissue Concentrations of Digoxin in an Autopsy Material," *Acta Pharmacologica et Toxicologica*, Vol. 34, 1974, pp. 385–390.

[5] Selesky, M., Spiehler, V., Cravey, R. H., and Elliot, H. W., "Digoxin Concentrations in Fatal Cases," *Journal of Forensic Sciences*, Vol. 22, No. 2, April 1977, pp. 409–417.

[6] Holt, D. W. and Benstead, J. G., "Postmortem Assay of Digoxin by Radioimmunoassay," *Journal of Clinical Pathology*, Vol. 28, 1975, pp. 483–486.

[7] Doherty, J. E., Perkins, W. H., and Flanigan, W. J., "The Distribution and Concentration of Tritiated Digoxin in Human Tissue," *Annals of Internal Medicine*, Vol. 66, 1967, pp. 116–124.

[8] Lang, D., Hofsteller, R., and von Bernuth, G., "Postmortem Tissue and Plasma Concentrations of Digoxin in Newborns and Infants," *European Journal of Pediatrics*, Vol. 128, 1978, pp. 151–161.

[9] Gorodischer, R., Jusko, W. J., and Yaffe, S. J., "Tissue and Erythrocyte Distribution of Digoxin in Infants," *Clinical Pharmacology and Therapeutics*, Vol. 19, 1976, pp. 256–263.

[10] Andersson, K. E., Bertler, A., and Wettrell, G., "Postmortem Distribution and Tissue Concentrations of Digoxin in Infants and Adults," *Acta Paediatrica Scandinavica*, Vol. 64, 1975, pp. 497–504.

[11] Gorodischer, R., Galil, A., and Kaplanski, J., "Brain, Myocardium and Plasma Concentrations and Toxicity of Digoxin in Newborn and Adult Rats," in *Proceedings of the 2nd International Congress of Clinical Pharmacology and Therapeutics*, Washington, DC, July 1983.

[12] DiGregorio, G. J., Piraino, A. J., Ruck, E. K., and Bassenches, P. J., "Investigation of Digoxin Quinidine and Disopyramide Interaction in Rats Utilizing Parotid Saliva, Blood and Other Tissues," *Journal of Pharmaceutical Sciences*, Vol. 71, 1982, pp. 211–213.

[13] Spiehler, V. R., Sedgwick, P., and Richards, R. G., "The Use of Brain Digoxin Concentrations to Confirm Blood Digoxin Concentrations," *Journal of Forensic Sciences*, Vol. 26, No. 4, Oct. 1981, pp. 645–650.

[14] Hastreiter, A. R. and VanDer Horst, R. L., "Postmortem Digoxin Tissue Concentration and Organ Content in Infancy and Childhood," *American Journal of Cardiology*, Vol. 52, 1983, pp. 330–335.

Address requests for reprints or additional information to
Gideon Koren, M.D.
The Division of Clinical Pharmacology
The Hospital for Sick Children
Toronto, Ontario M5G 1X8 Canada

ORIGINAL CONTRIBUTION
digoxin, serum levels

Serum Digoxin Levels and Mortality in 5,100 Patients

A retrospective study of 5,100 patients on digoxin, with a four-week follow up after digoxin levels were measured, was done to determine the mortality rate. A significant increase in mortality was correlated with an increasing serum digoxin level, up to 50% at a level of 6.0 ng/mL and more. Clinical toxicity was suspected in only 0.25% of all patients on digoxin, although almost 10% had levels above the therapeutic range. Deliberate digoxin overdoses were fatal in 50% of cases. This study shows a correlation between increasing digoxin levels and increasing mortality rates. We recommend the use of serum digoxin measurements to identify those asymptomatic patients with elevated levels. The physician should seriously consider the indications for initiating or continuing digoxin treatment in any patient because of an increased mortality in patients with levels of more than 1.0 ng/mL. [Ordog GJ, Benaron S, Bhasin V, Wasserberger J, Balasubramanian S: Serum digoxin levels and mortality in 5,100 patients. Ann Emerg Med January 1987;16:32-39.]

Gary J Ordog, MD, FACEP, FAACT
Steve Benaron, MD, FACEP
Vijay Bhasin, MD, FACC
Jonathan Wasserberger, MD, FACEP
Subramaniam Balasubramanium, MD
Los Angeles, California

From the Department of Emergency Medicine, King/Drew Medical Center, and the Charles R Drew Postgraduate Medical School, Los Angeles, California.

Received for publication July 30, 1984. Revisions received November 11, 1985, and February 10, 1986. Accepted for publication May 7, 1986.

Address for reprints: Gary J Ordog, MD, FACEP, FAACT, Box 219, 12021 South Wilmington, Los Angeles, California 90059.

INTRODUCTION

Cardiac glycosides have been used for congestive heart failure and certain cardiac arrhythmias for more than 200 years.[1] Digitalis affects the Na+/K+ ATPase, thus influencing plasma membrane transport receptors on cardiac cells, providing an explanation for at least some of its action.[2,3] A narrow margin exists between therapeutic and toxic doses of digoxin, resulting in a high incidence of digoxin toxicity in clinical practice.[4] Alterations in cardiac rhythm, inotropism, and cardiac electrophysiology, and such extracardiac manifestations of digitalis action as gastrointestinal and central nervous system symptoms are dose related.[5-9] Increasing digoxin dosage increases serum digoxin concentrations so that statistically a relationship is expected between dosage and clinical state.[10]

A review of the literature reveals studies showing a relationship between serum digoxin level and clinical state in a total of more than 1,000 patients.[11-39] Combining the data from all preexisting studies, the mean serum or plasma digoxin level of all nontoxic patients was 1.4 ng/mL, while the mean level of clinically toxic patients was two to three times higher.[4] The mean level in toxic compared with nontoxic patients differed statistically in most studies, but there was a significant overlap between the two groups.[4] This difference is far more pronounced in the prospective than in the retrospective studies.[11,40-46]

No single serum or plasma digoxin level can be selected that clearly separates toxic and nontoxic states in the usual clinical setting. This is predictable based on the many factors affecting individual sensitivity to the toxic effects of digitalis glycosides.

Our purpose was to determine if elevated digoxin levels correlate with an increased mortality rate. Our retrospective study examined patients on digoxin to further delineate the usefulness of serum digoxin level determination and clinical indications for drawing a digoxin level.

METHODS

The King/Drew Medical Center has determined serum digoxin levels by radioimmunoassay for 12 years. Routine digoxin levels are drawn on every patient who is on digoxin when seen in all medical units, emergency depart-

PLAINTIFFS' EXHIBITS 012604

9-22

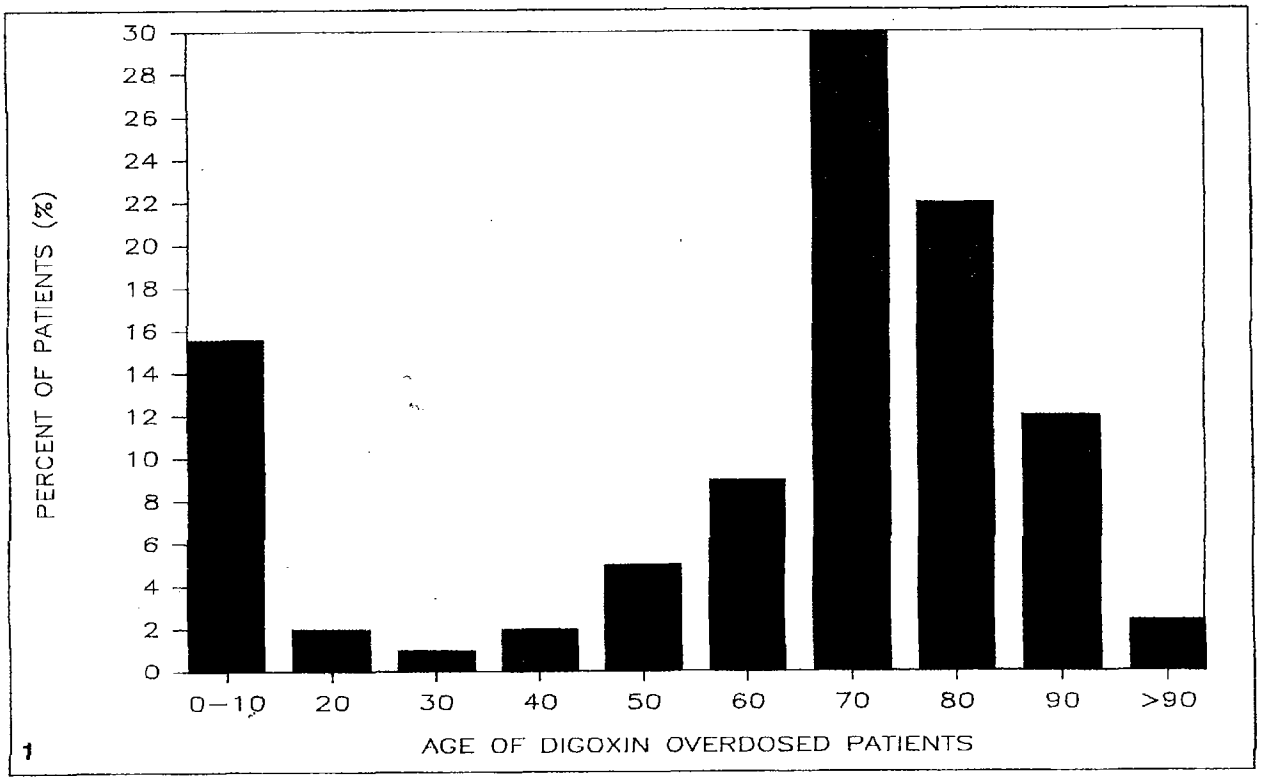


FIGURE 1. *Age distribution of digoxin overdose.*

ments, and outpatient clinics. Levels are drawn at least six hours after the last oral dose of digoxin. The study patients included all those who were treated in the hospital, including the ED and various clinics. The logbooks showing all levels measured were reviewed; from these the charts of patients were obtained. The hospital charts were reviewed to determine mortality rates and other clinical data on all persons treated as both inpatients and outpatients.

The data of patients with values of 0.0 to 1.0 ng/mL and 1.1 to 2.0 ng/mL were compared to the same data and motality rates of matched general medical patients admitted to the same institution who were not on digoxin. Excluded were surgical, obstetrical, orthopedic, gynecologic, and all other nonmedical specialty patients. The control group was matched for age, sex, race, and associated diagnoses and prognosis.

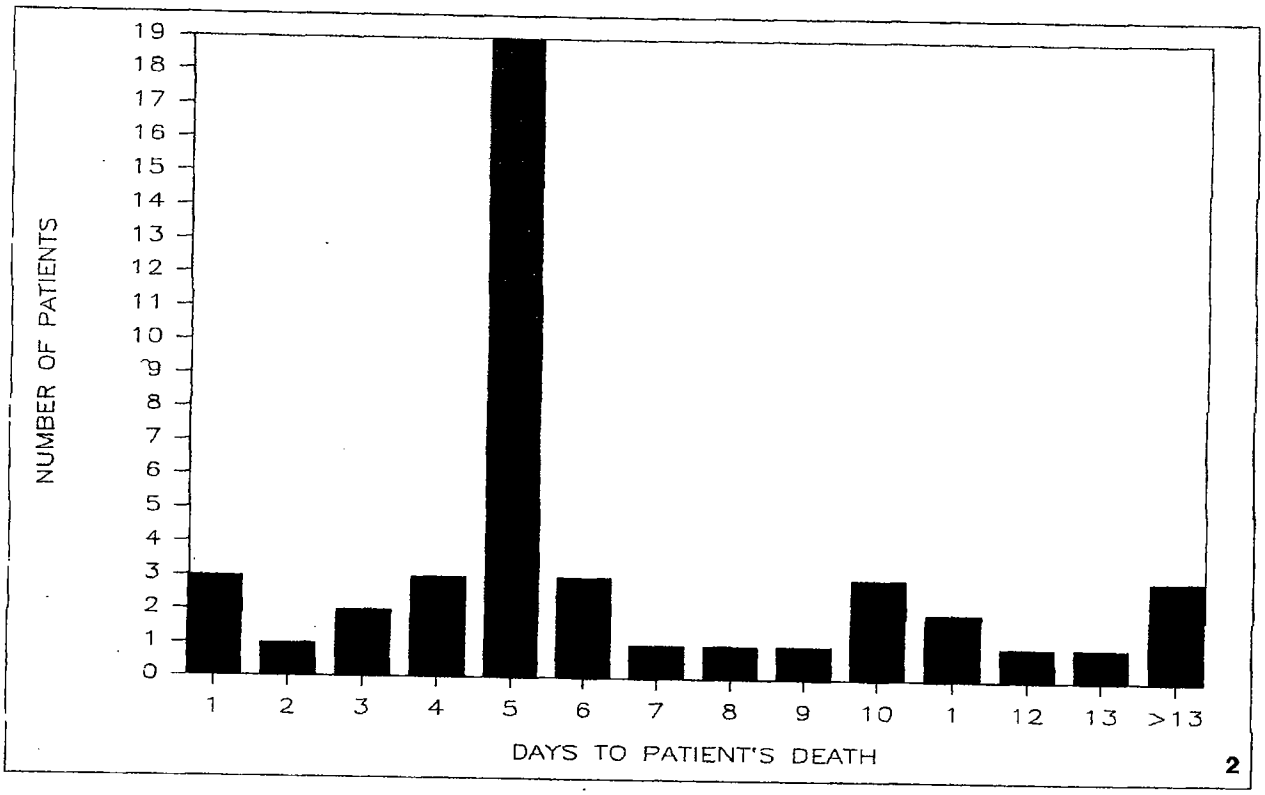
Therapeutic levels were defined as those from 1.0 to 2.0 ng/mL. The levels above 6.0 ng/mL could not be measured and were reported as being "greater than 6.0." After 1980, the laboratory was able to measure values of digoxin up to 12.0 ng/mL, and these were reported as "greater than 12.0."

The mortality rate for outpatients was determined by reviewing the outpatient charts and by comparing these patient names to deaths listed in the coroner's office logbook. The name of any patient dying within four weeks of the study time who had not moved out of the county should have been detected by this method.

Digoxin levels were measured by ^{125}I digoxin radioimmunoassay using the ARIA-HT® system until 1980 and the AIRA-II® system after 1980 (Becton Dickinson Immunodiagnostics, Salt Lake City, Utah).

The following clinical data were collected from the hospital chart on each patient with a level above the therapeutic range: sex, age, race, diagnosis, ancillary medications, serum potassium levels, treatment, length of hospitalization, digoxin dosage, adjustment of digoxin dose, length of time from last digoxin dose to when serum was taken for measurement, complications, and presence of clinical signs of digoxin toxicity. Admission requirements for all patients included complete blood count, electrolytes, BUN, creatinine, chest radiograph, and ECG. Mortality was considered death occurring up to four weeks from the time the digoxin level was measured; for inpatients not taking digoxin (control group), mortality was considered death within four weeks from the date of hospital admission.

The patients' levels of digoxin above the therapeutic range of 2.0 ng/mL were divided into three subgroups according to the digoxin level: 2.1 to 4.0 ng/mL, 4.1 to 6.0 ng/mL, and higher than 6.0 ng/mL. Only the highest level recorded was used in this study if several were drawn. These groups were compared for all of the variables, and were compared to the general medical admission patients who were matched for the characteristics of age, sex, and length of hospital admission.



They were compared to those with levels of 0 to 1.0 ng/mL and 1.1 to 2.0 ng/mL, and for statistical differences by the Student t test, analysis of variance. The program BMDP2V Analysis of Variance and Covariance with Repeated Measures at the Health Sciences Computing Facility of UCLA Medical Center was used, assisted by the Department of Biostatistics, School of Public Health, through members of the Statistics Consulting Laboratory. The level of significance for all tests was $P < .05$.

RESULTS

Mortality

The study consisted of 6,133 levels from 5,100 patients between 1972 and 1982. Clinical assessment was performed by more than 1,000 resident physicians under the guidance of approximately 25 attending physicians in the departments of internal medicine and emergency medicine. Adherence to the policy of doing digoxin levels was more than 50%; that is, at least 50% of all patients taking digoxin and seen at the hospital for any reason had digoxin levels drawn, even when there were no signs of digoxin toxicity. The adherence was more than 95% for inpatients, more than 90% for those seen in the ED, but less than 50% for those seen in clinics for problems unrelated to digoxin toxicity (eg, those seen for minor trauma).

Nine percent of patients (460) had levels of 2.1 ng/mL or more. Sixty-four percent (3,184) of the patients evidenced therapeutic levels of 1.1 to 2.0 ng/mL. Twenty-seven percent (1,366) had levels of 0 to 1.0 ng/mL.

Among the elevated laboratory levels (more than 2.0 ng/mL), there were two definite age groups — pediatric and adult. The mean ages statistically showed a bimodal distribution. The mean adult age was 66.8 years (SD, 31.0 years), and the pediatric group had a mean age of 12.4 months (SD, 28 months). Overall the mean age was 55.1 years (SD, 29.0 years). Eighty-three (18%) were pediatric patients, and 377 (82%) were more than 18 years old (Figure 1).

Of the 460 patients with digoxin levels above the therapeutic range, 349 (76%) were admitted to the hospital and 111 (24%) were evaluated and treated as outpatients in the ED, usually prior to the physician's receipt of the digoxin level. Hospitalized patients with levels of more than 2.1 ng/mL spent a mean of 12.1 days in hospital (SD, 17.1 days). The mean time to death for patients in this group who died was five days (SD, 3.1 days) (Figure 2).

FIGURE 2. *Number of days to patient's death.*

General medical patients not on digoxin had a mortality rate of 2.0%. The group of treated patients with levels of 0 to 1.0 ng/mL also had a mortality rate of 2.0%. Those in the group with levels ranging from 1.1 to 2.0 ng/mL had a mortality rate of 5% (Table 1, Figure 3).

Among the 460 patients with levels of 2.1 ng/mL and more, 44 (9.6%) died. Of these 460 patients, 14 of the 111 outpatients died (mortality rate, 13%) and 30 of the 349 inpatients died (mortality rate, 9%).

Fourteen adult patients taking di-

TABLE 1. *Mortality rate for digoxin levels*

Digoxin Level	No. Patients	No. Deaths	Mortality Rate (%)*	% Clinical Digoxin Toxicity
0†	1,000‡	20	2.0	0
0-1.0	1,366	26	2.0	0
1.1-2.0	3,184	159	5.0	0
2.1-4.0	409	36	8.6	3
4.1-6.0	40	3	7.5	0
>6.0	11	5	50.0	0

*For patients not taking digoxin, this time interval was a four-week period after hospital admission. For those taking digoxin, it was a four-week period after digoxin level was measured.

†Patients were matched hospital admissions who were not taking digoxin (excluded all surgical, obstetrical, and gynecological patients, but included ICU and CCU patients).

‡The log was reviewed for 1,000 patients to obtain a general medical admission mortality rate for patients not taking digoxin.

TABLE 2. *ECG changes noted on retrospective examination**

Therapeutic digoxin levels — 15% had ECG changes
Elevated digoxin levels — 30% had ECG changes
Elevated digoxin levels — 14% had new-onset abnormalities

ECG Abnormality	New Onset	Old Changes	Mortality Rate (%)
Atrial fibrillation		46	0
PVCs with bradycardia	12		0
Pacemaker driven		12	0
PAT with block	20		25
First-degree heart block	12		42
Sinus arrest	5		60
Complete heart block	10		100
All new abnormalities	62		40
All old abnormalities	70		8

*Excluding acute myocardial infarction changes.

goxin were admitted to the ED in cardiopulmonary arrest; five were resuscitated and survived to be discharged home. The mean digoxin level of these patients was 2.43 ng/mL. Sixteen hospitalized infants receiving digoxin suffered cardiac arrest, of whom four survived. The mean digoxin level of these infants was 3.8 ng/mL; all had congenital heart disease.

Digitalis Levels

The mean level for all patients with levels above 2.1 ng/mL was 2.88 ng/mL (SD, 0.99). The outpatients' mean level was 2.63 (SD, 0.50) while the inpatients' level, 3.06 (SD, 1.3), was higher. The mean digoxin level of hospitalized patients with elevated levels who died was 2.98 (SD, 1.32). The mean level at the time of death actually had increased to 3.86 (SD, 1.96). Two-thirds of the patients who died in hospital had rising digoxin levels prior to death, which was statistically different from the initial to the final level ($P < .05$). One-third of these patients who died had levels above the measurable limit at the time of death (6.0 ng/mL from 1972 to 1980, and 12.0 ng/mL from 1980 to 1982).

The other 15 had either steady or decreasing levels at the time of death. The conditions associated with these rising levels prior to death included congenital heart disease with congestive failure (22%), dehydration (18%), acute congestive heart failure and poor perfusion (9%), renal failure (2%), suicidal overdose (1%), and unknown causes (36%).

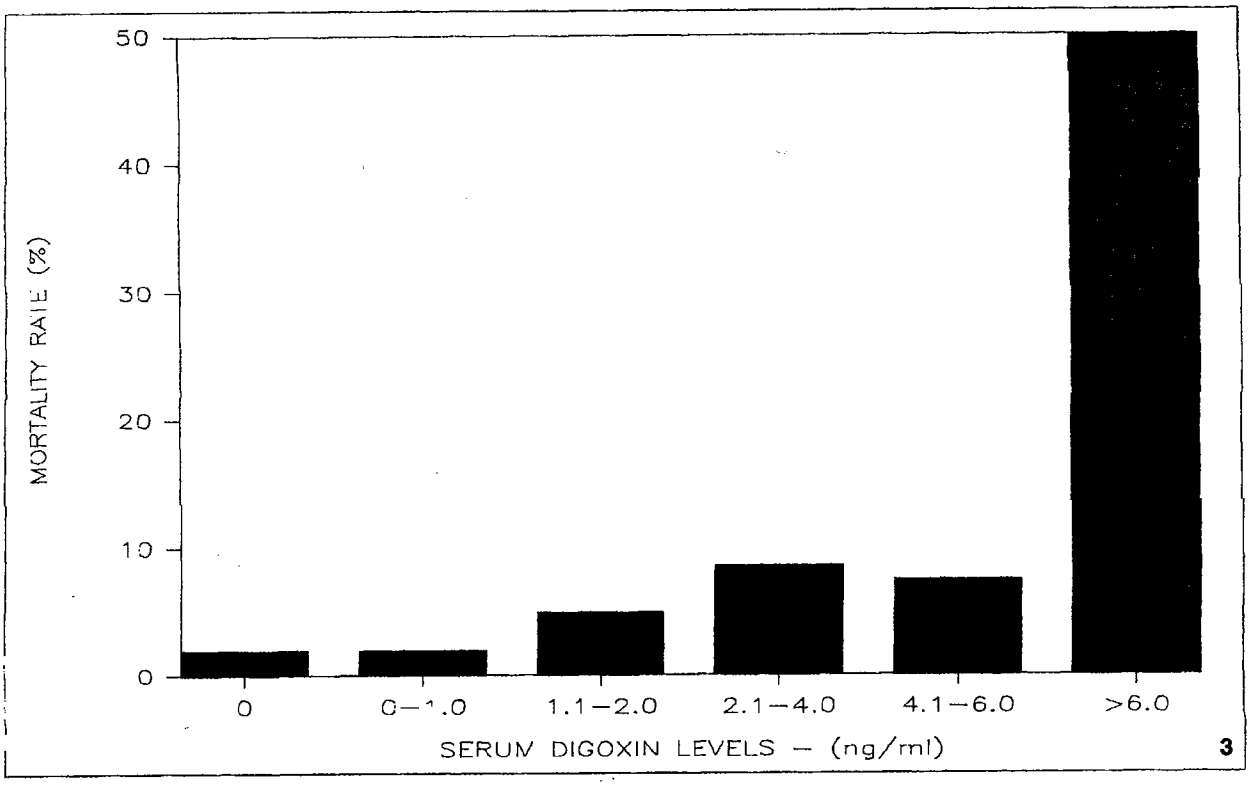
Forty patients (8.6% of all elevated levels) had levels of 4.1 to 6.0 ng/mL. Three died (Figure 4). Eleven patients (2.4% of all elevated levels) had levels of more than 6.0 ng/mL; their mortality rate was 50%. Pulses ranged from 30 to 60 per minute (mean, 46). None had a clinical diagnosis of digoxin toxicity. The only clue was the elevated digoxin level.

Clinical Digoxin Toxicity

Of 460 patients with elevated levels, only 13 were diagnosed by the examining physician as having digoxin toxicity prior to receiving the digoxin

PLAINTIFFS' EXHIBITS 012607

9-25



level. The diagnostic impressions of more than 1,000 physicians were reviewed to show that only 13 patients had clinical evidence of digoxin toxicity; all were hospitalized, but none died. The mean initial level of these patients was 2.66 ng/mL (range 2.53 to 3.54). The potassium level ranged from 3.6 to 6.5 mEq/L; no patient was hypokalemic. None was being digitalized at the time of diagnosis. Serum BUN levels were normal except in 2% of patients who had acute renal failure and 9% of patients who had dehydration with BUN levels ranging from 20 to 60 mg/100 mL. Renal failure did not statistically worsen the prognosis due to elevated digoxin levels compared to patients with similar digoxin levels and normal BUNs, as long as the digoxin was discontinued.

All charts were reviewed for clinical and ECG signs of digoxin toxicity (Table 2). Sixty-five patients had new ECG changes consistent with digoxin toxicity (14% of all patients with elevated digoxin levels). The mean level of these patients was 3.82 ng/mL. The mortality rate was 15%, which was statistically different ($P = .02$) from the group that had been diagnosed prospectively as having digoxin toxicity. When clinical digoxin toxicity was diagnosed early in the patient's management, the mortality rate was significantly decreased ($P = .04$) from that for patients in whom the diagnosis was made by delayed digoxin level. This resulted from the early management of cardiac arrhythmias and intensive care monitoring of these patients compared with those in whom the digoxin levels provided the only evidence of toxicity (Table 2).

FIGURE 3. *Mortality rate vs digoxin level.*

Electrocardiograms

Each hospitalized patient had an ECG prior to admission. Thirty percent of patients with elevated digoxin levels had abnormal ECGs, versus 15% for those with low or therapeutic digoxin levels ($P = .04$). Of these, 57% were preexistent abnormalities. Forty-six patients had preexistent atrial fibrillation and all survived, with heart rates varying from 46 to 90 beats per minute. Twenty patients had paroxysmal atrial tachycardia, which resulted in death in five. Ten patients had a new onset of complete heart block, and all died despite pacemaker insertion. Three of 5 patients with asystole died. Five patients who demonstrated only first-degree heart block died, while seven others lived. Eleven patients had premature ventricular contractions with either an underlying normal sinus rhythm or a sinus bradycardia, and all lived. Twelve patients with demand pacemaker rhythms lived. The mortality rate with elevated digoxin levels and preexisting ECG abnormalities was 8%, compared with 40% for patients with elevated digoxin levels and new ECG abnormalities. The difference was statistically significant ($P = .035$) (Table 2).

Digoxin Level and Mortality Rate

The mortality rate of matched patients not on digoxin who were admitted to the medical service during a

four-week period following admission was 2%. Those in the group taking digoxin who had levels of 0 to 1.0 ng/mL also had a mortality rate of 2.0% for a four-week period subsequent to the time the digoxin measurement was made. Those patients with a level ranging from 1.1 to 2.0 ng/mL had a four-week mortality rate of 5.0%. The group with values ranging from 2.1 to 4.0 ng/mL had an 8.6% mortality rate; the group ranging from 4.1 to 6.0 ng/mL had a 7.9% mortality rate; and the group with digoxin levels of more than 6.0 ng/mL had a 50% four-week mortality rate. Each group on digoxin had a statistically different mortality rate, except for those with levels of 2.1 to 4.0 ng/mL and 4.1 to 6.0 ng/mL.

No patient with a level of more than 6.0 ng/mL was clinically diagnosed as having digitalis toxicity. The diagnosis was made only on the laboratory measurements. This is compared with the group with levels of 2.1 to 4.0 ng/mL in which 13 patients (3%) were diagnosed clinically as having digoxin toxicity. Among all patients taking digoxin in this study (N = 5,100), only 0.25% were diagnosed as having digoxin toxicity.

Deliberate Digitalis Overdose

Five patients with elevated levels had taken a deliberate overdose, either as a suicidal gesture or a serious suicide attempt. Both patients with levels exceeding 6.0 ng/mL died. The other three survived with no further morbidity after four weeks of follow up. Cardiac glycoside-specific antibody treatment[4,5] was not available for these patients. Pacemakers were inserted in two who had clinically significant bradycardia.

DISCUSSION

Because our study was retrospective, and only 50% of the patients on digoxin may have been evaluated, we were unable to compare the actual incidence of digoxin toxicity with the laboratory diagnosis of elevated digoxin levels measured by radioimmunoassay in a large teaching hospital. Most prospective studies[11-39] have shown a far higher rate of clinical digoxin toxicity than have retrospective studies,[11,40-46] probably because researchers using a prospective study design are specifically seeking signs of digoxin toxicity.

In our study, detection of clinical digoxin toxicity was low — only 2.8% of those with elevated levels when initially examined and 14% on retrospective review of the charts (consistent with other retrospective studies[11,40-46]). We believe that knowledge of the digoxin levels had no influence on the clinical diagnosis because laboratory levels were usually unavailable for 24 to 72 hours after being drawn; thus, the examining physician had to make the diagnosis based on clinical evaluation.

There was a poor correlation between signs and symptoms, ECG changes, and serum digoxin levels. This would not be significant except for the fact that there also was a marked increase in mortality with increasing digoxin levels, reaching 50% mortality above 6.0 ng/mL. No patient in that group was clinically suspected of having digoxin toxicity.

Prospective clinical assessment revealed digoxin toxicity in only 2.8% of those patients with elevated serum digoxin levels. Retrospective ECG evaluation showed that 30% of patients with elevated levels had abnormal ECGs, compared with 15% of patients with low or therapeutic levels. ECG changes alone were not associated with increased mortality. But by combining new ECG abnormalities with elevated digoxin levels, statistically increased mortality rates ($P = .04$) can be expected for patients with new ECG abnormalities. The new ECG abnormalities associated with the highest mortality rates were paroxysmal atrial tachycardia with block, complete heart block, sinus arrest, and new-onset first-degree heart block. Our conclusion about the use of ECGs in the diagnosis of digoxin toxicity is that ECGs by themselves can be useful if new abnormalities are present, but their value is greatly increased when combined with a digoxin level to predict the mortality rate.

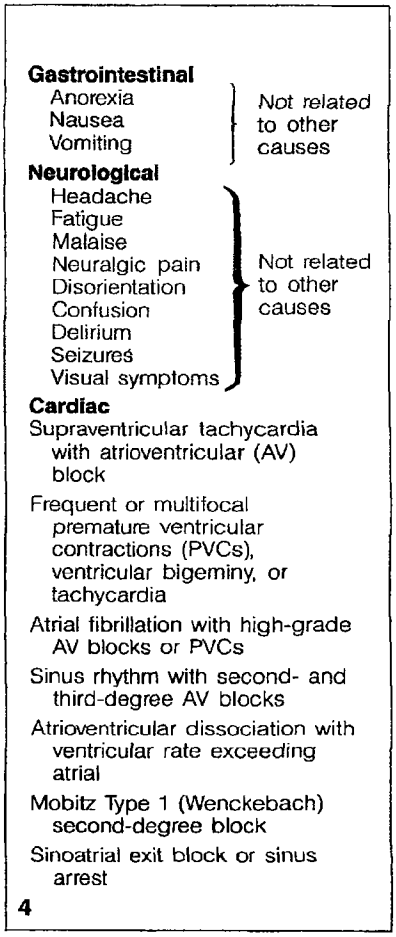
Digoxin Levels and Mortality Rates

Of all hospitalized patients who had digoxin levels above the laboratory therapeutic range, 10% died within four weeks, compared with 2% among matched hospital medical patients not taking digoxin. Therefore, elevated digoxin levels correlate positively with increased mortality, with or without clinical toxicity, to $P < .05$. Finding a matched control group with similar diagnoses who are not taking digoxin may not be an entirely appropriate control.

Of all the patients on digoxin, only 0.25% were thought clinically to have digoxin toxicity, but within a four-week period after the digoxin level was measured, 5% of the patients had died. By far the highest percentage of deaths occurred in the group of patients with levels above 2.0 ng/mL.

Our study revealed a significant correlation between serum digoxin level and mortality rate, with increasing levels of serum digoxin being associated with even greater mortality.

FIGURE 4. *Symptoms and signs of digoxin toxicity. Criteria for presence of digoxin toxicity include the disappearance of the above signs and symptoms when the digoxin was withheld.*



PLAINTIFFS' EXHIBITS 012609

9-27

Some authors have concluded that despite the overlap in levels between the toxic and nontoxic patients, the use of serum digoxin levels can reduce the incidence of digoxin toxicity.[40-51] Others recommend that serum digitalis levels be used in particular situations, for instance, in the absence of an adequate history, fluctuating renal function, suspected malabsorption, and when preparations of uncertain bioavailability are used.[5,40-51] More generally, the measurement of serum cardiac glycoside concentration has been recommended whenever an unanticipated response to these drugs is encountered (either suspected toxicity or absence of therapeutic response).[5]

Another use has been to estimate the patient's compliance in taking medication.[5] To our knowledge, no author has yet recommended routine digoxin measurements when clinically evaluating a patient or digoxin. Moreover, we could find no study that compared the mortality rate of various digoxin levels in large groups of patients.

The use of serum digoxin concentration measurements to guide therapy has been proven to reduce the incidence of digoxin toxicity.[5,46] In our study, because only a small number manifested overt clinical digoxin toxicity with elevated levels and because of the drastic increase in mortality with increasing levels of digoxin above laboratory-defined therapeutic ranges, we recommend more frequent monitoring of digoxin levels when any patient is taking digoxin or is suspected of taking it. Routine monitoring may detect those patients with elevated levels who are at greatest risk of dying during the ensuing four weeks.

Serum potassium levels had no correlation with elevated digoxin levels, although the mean potassium level was statistically elevated ($P = .045$) in these patients compared with their controls. This is surprising considering that almost invariably patients taking digoxin are also on a diuretic. It is likely that elevation of serum potassium is a consequence of inhibition of Na+/K+ ATPase throughout the body, with consequent impairment of monovalent cation transport across cell membranes. Elevations of serum potassium have been shown to be associated with worsening prognosis after massive doses, usually of digitoxin.[52] Refractory hyperkalemia can occur at extremely high digoxin doses and serum concentrations.[53-55] Ours is the first report to show a statistically elevated serum potassium level in overdosed patients.

CONCLUSIONS

Twenty-four-hour rapid availability of serum digoxin assay may benefit outpatients on this drug because they have a higher mortality rate than do inpatients when the level is above 2.0 ng/mL. This could prevent patients on digoxin from being discharged on the same dosage when they actually are above the therapeutic range, with no signs of digoxin toxicity. We recommend that these patients be monitored closely while their levels return to the therapeutic range. The cost of a digoxin level by radioimmunoassay at the time of this study was $32, which is less than many other laboratory tests that are routinely ordered in these patients. If an immediate digoxin level were available, we believe that it would be cost effective in reducing the mortality rate.

There is a statistically decreased mortality over a four-week period for patients who have even lower levels of digoxin than those within the therapeutic range. Patients with serum levels of 0 to 1.0 ng/mL had a mortality rate of only 2.0%, compared to 5.0% for those in the therapeutic range of 1.0 to 2.0 ng/mL. This should make the physician seriously consider the indications for digitalization. If the indication for digitalization is questionable, if the therapeutic response is minimal, or if the patient no longer requires digoxin, it should be discontinued. The matched control group not receiving digoxin had a four-week mortality rate of 2.0%, which was identical to that of 1,366 patients who had subtherapeutic levels of digoxin. One may infer from these data that patients who are inadequately digitalized probably do not require digitalization, for their mortality does not differ from that of the nondigitalized population.

The potential of digoxin antibodies in the treatment and reduction of mortality of asymptomatic overdose patients with levels above 6.0 ng/mL appears promising and needs further evaluation.[56-59] Further prospective studies also are required to discover whether the rapid availability of serum digoxin levels will decrease the mortality rates.

REFERENCES

1. Withering W: An account of the foxglove, in Willus FA, Keys TE (eds): *Cardiac Glycosides*. St Louis, CV Mosby Co, 1941, p 232.

2. Caldwell PC, Keynes RD: The effect of ouabain on the efflux of sodium from a squid giant axon. *J Physiol* 1959;148:8P-9P.

3. Hoff JF: The red cell membrane and the transport of sodium and potassium. *Am J Med* 1966;41:666.

4. Smith TW, Curtman GD, Green LH: The use and misuse of digoxin blood levels, in Hurst JW (ed): *The Heart*. New York, McGraw-Hill Book, Inc, 1982, p 75.

5. Braunwald E (ed): *Heart Disease: A Textbook of Cardiovascular Medicine*. Philadelphia, WB Saunders Co, 1980, p 532.

6. Moe GK, Farah AE: Digitalis and allied cardiac glycosides, in Goodman LS, Gilman A (eds): *The Pharmacological Basis of Therapeutics*, ed 5. New York, Macmillian Co, 1975, p 652.

7. Doherty JE, Perkins WH, Flanigan WJ: The distribution and concentration of tritiated digoxin in human tissues. *Ann Intern Med* 1967;66:116-120.

8. Barr I, Smith TW, Klein MD, et al: Correlation of the electrophysiologic action of digoxin with serum digoxin concentration. *J Pharmacol Exp Ther* 1972;180:710-722.

9. Doherty JE, Perkins WH: Tissue concentration and turnover of tritiated digoxin in dogs. *Am J Cardiol* 1966;17:47-52.

10. Gullner HG, Stinson EB, Harrison DC, et al: Correlation of serum concentrations with heart concentrations of digoxin in human subjects. *Circulation* 1974;50:653-655.

11. Beller BA, Smith TW, Abelmann WH, et al: Digitalis intoxication: A prospective clinical *study with serum level correlations. N Engl J Med* 1971;284:989-997.

12. Bertler A, Redfors A: Plasma levels of digoxin in relation to toxicity. *Acta Pharmacol Toxicol* 1971;29(Suppl 3):281-287.

13. Bertler A, Gustafson A, Ohlin P, et al: Digoxin intoxication and plasma glycoside levels, in Storestein O (ed): *Symposium on Digitalis*. Oslo, Gyldendal Norsk Forlag 1973, p 300.

14. Brooker G, Jelliffe RW: Serum cardiac glycoside assay based upon displacement of ^{3}H-ouabain from Na-K ATPase. *Circulation* 1971; 45:20-36.

15. Burnett GH, Conklin RL: The enzymatic assay of plasma digoxin. *J Lab Clin Med* 1971;78:779-784.

16. Curruthers SG, Kelly JG, McDevitt DC: Plasma digoxin concentration in paticnts on admission to hospital. *Br Heart J* 1974;3:707-712.

17. Chamberlain DA, White RJ, Howard MR, et al: Plasma digoxin concentration in patients with atrial fibrillation. *Br Med J* 1970;3:429-432.

18. Doering W, Konig E, Sturm W: Digitalis intoxication: Specificity and significance of cardiac and extracardiac symptoms. Part I: Patients with digitalis induced arrhythmias. *Z Kardiol* 1977;66:121-126.

19. Evered DC, Chapman D: Plasma digoxin concentrations and digoxin toxicity in hospital patients. *Br Heart J* 1971;33:540-545.

20. Fogelman AM, LaMont JT, Finkelstein S, et

PLAINTIFFS' EXHIBITS 012610

9-28

al: Fallibility of plasma digoxin in differentiating toxic from non-toxic patients. *Lancet* 1971;2:727-729.

21. Grahame-Smith DG, Everest MS: Measurement of digoxin in plasma and its use in diagnosis of digoxin intoxication. *Br Med J* 1969;1:286-289.

22. Hayes CJ, Butler VP, Gersony WM: Serum digoxin studies in infants and children. *Pediatrics* 1973;52:561-568.

23. Hoeschen RJ, Proveda V: Serum digoxin by radioimmunoassay. *Can Med Assoc J* 1971; 105:170-173.

24. Howard D, Smith CI, Stewart G, et al: A prospective survey of the incidence of cardiac intoxication with digitalis in patients being admitted to the hospital and correlation with serum digoxin levels. *Aust NZ J Med* 1973;3:279-287.

25. Huffman DH, Crow JW, Pentikainen P, et al: Association between clinical cardiac status, laboratory parameters, and digoxin usage. *Am Heart J* 1976;91:28-34.

26. Isisalo E, Dahl M, Sundquist H: Serum digoxin in adults and children. *Int J Clin Pharmacol* 1973;7:219-223.

27. Johnston DI, Pinkus NB, Down M: Plasma digoxin levels in digitalized and toxic patients. *Med J Aust* 1972;1:863-866.

28. Kraula R, Tanagi R, Hastreider AR, et al: Digoxin intoxication in infants and children. *J Pediatr* 1974;84:265-269.

29. Lader S, Bye A, Marsdin P: The measurement of plasma digoxin concentration: A comparison in two methods. *Eur J Clin Pharmacol* 1972;5:22-25.

30. McCredi RM, Chia BL, Knight PW: Infants versus adult plasma digoxin levels. *Aust NZ J Med* 1974;4:223-227.

31. Morrison J, Killip T, Stason WB: Serum digoxin in patients undergoing cardiopulmonary bypass. *Circulation* 1970;42:110-115.

32. Oliver GC, Parker BM, Parker CW: Radioimmunoassay for digoxin, technique and clinical application. *Am J Med* 1971;51:186-189.

33. Park HM, Chen IW, Manitassas GT, et al: Clinical evaluation of radioimmunoassay digoxin. *J Nucl Med* 1973;14:531-533.

34. Ritzmann LW, Bangs CC, Coiner D, et al: Serum glycoside levels by rubidium assay. *Arch Intern Med* 1973;132:823-826.

35. Scherrmann JM, Bourdon R: Dosage de la digoxine par methode radioimmunologique. *Eur J Toxicol* 1976;9:133-136.

36. Singh RB, Rai AN, Srivastav DK, et al: Radioimmunoassay of serum digoxin in relation to digoxin intoxication. *Br Heart J* 1975; 37:619-621.

37. Smith TW, Haber E: Digoxin intoxication: The relationship of clinical presentation to serum digoxin concentration. *J Clin Invest* 1970;49:2377-2386.

38. Waddorff S, Buch J: Serum digoxin and empiric methods in identification of digitoxicity. *Clin Pharmacol Ther* 1978;23:19-24.

39. Lukas DS, Peterson RW: Double isotope dilution derivative assay of digitoxin in plasma, urine and stool of patients maintained on the drug. *J Clin Invest* 1966;45:782-784.

40. Morrison J, Killip T: Radioimmunoassay of digitoxin. *Clin Res* 1970;14:782-784.

41. Peters U, Hausamen TU, Grosse-Brockhoff F: Serial tests of serum digitoxin levels during digitoxin treatment. *Dtsch Med Wochenschr* 1974;99:1701-1704.

42. Rasmussen K, Jervell J, Storstein O: Clinical use of a bioassay of serum digitoxin activity. *Eur J Clin Pharmacol* 1971;3:236-238.

43. Weissel M, Fritzsche H, Fuchs G: Salivary electrolytes and serum digoxin in the assessment of digitalis intoxication. *Wien Klin Wochenschr* 1976;88:455-458.

44. Whiting B, Summer DJ, Goldbert A: An assessment of digoxin radioimmunoassay. *Scot Med J* 1973;18:69-72.

45. Zeegers JJW, Maas AHJ, Willebrads AF, et al: The radioimmunoassay of plasma digoxin. *Clin Chim Acta* 1973;44:109-111.

46. Duhme DW, Greenblatt DJ, Koch-Weser J: Reduction of digoxin toxicity associated with measurement of serum levels. *Ann Intern Med* 1974;80:516-519.

47. Bentley JD, Burnett GH, Conklin RL, et al: Clinical application of serum digitoxin levels: A simplified plasma determination. *Circulation* 1970;41:67-70.

48. Chiche P, Baligadoo S, Larvelle P, et al: Intoxications digitaliques et deviations de l'activite therapeutique de la digitaline. *Coeur Med Intern* 1976;15:249-253.

49. Dessaint JP: Dosage radio-immunoloique des digitalique [digitoxine et digoxine] dans le sang. *Lille Med* 1974;19:156-158.

50. Hillestad L, Hansteen V, Hatle L, et al: Digitalis intoxication, in Storstein O (ed): *Symposium on Digitalis*, Oslo, Gyldendal Norsk Forlag, 1973, p 281.

51. Smith TW, Butler VP Jr, Haber T: Determination of therapeutic and toxic serum digoxin concentrations by radioimmunoassay. *N Engl J Med* 1969;281:1212-1214.

52. Smith TW, Willerson JT: Suicidal and accidental digoxin ingestion: Report of five cases with serum digoxin level correlations. *Circulation* 1971;44:29-31.

53. Asplund J, Edlag O, Mogensen L, et al: Four cases of massive digitalis poisoning. *Acta Med Scand* 1971;189:293-294.

54. Citrin D, Stevenson IH, O'Malley K: Massive digoxin overdose: Observations on hyperkalemia and plasma digozin levels. *Scott Med J* 1972;17:275-278.

55. Gaultier M, Fournier E, Efthymiou M, et al: Intoxication digilique aigue (70 observations). *Bull Soc Med Hop Paris* 1968;119:247-251.

56. Smith TW, Haber E, Yeatman L, et al: Reversal of advanced intoxication with FAB fragments of digoxin specific antibodies. *N Engl J Med* 1976;15:797-800.

57. Murphy DJ Jr, Bremner WF, Haber E, et al: Massive digoxin poisoning treated with FAB fragments of digoxin specific antibodies. *Pediatrics* 1982;70:468-471.

58. Zucker AR, Lacina SJ, Das Gupta DS, et al: FAB fragments of digoxin specific antibodies used to reverse ventricular fibrillation induced by digoxin ingestion in a child. *Pediatrics* 1982;70:468-471.

59. Smith TW, Butler VP Jr, Haber E, et al: Treatment of life threatening digitalis intoxication with digoxin specific FAB antibody fragments. Experience in 26 cases. *N Engl J Med* 1982;307:1357-1362.

PLAINTIFFS' EXHIBITS 012611

9-29

Int J Leg Med (1992) 105 : 149–154

International Journal of
Legal Medicine
© Springer-Verlag 1992

Measurement of digitalis-glycoside levels in ocular tissues:

A way to improve postmortem diagnosis of lethal digitalis-glycoside poisoning? I. Digoxin*

S. Ritz, P. Harding, W. Martz, H. W. Schütz, and H.-J. Kaatsch

Institut für Rechtsmedizin, Christian-Albrechts-Universität zu Kiel, Arnold-Heller-Strasse 12, W-2300 Kiel, Federal Republic of Germany

Received June 6, 1992 / Received in revised form August 13, 1992

Summary. Prompted by animal studies reporting the accumulation of digitalis-glycosides in ocular tissues, we investigated whether measurement of digoxin levels in human ocular tissues can improve the postmortem diagnosis of lethal digoxin intoxication. Digoxin was measured in the vitreous humor and choroid-retina of patients who had received in-patient treatment with digoxin prior to death (therapeutic group) and in a single case of suicidal intoxication. The results were compared with the digoxin levels in the femoral vein blood, myocardium, kidney and liver, and evaluated in light of the medical history of each patient. In the therapeutic group the mean digoxin level was higher in the choroid-retina than in other tissues and body fluids. The range of variation in levels in the choroid-retina following therapeutic doses was comparable to that in the other tissues. An extremely high level of digoxin was present in the choroid-retina in the case of suicidal intoxication. In all cases, levels in the vitreous humor were very low compared to those in the choroid-retina. Hence, it is unlikely that significant distortion of choroid-retinal levels occurs due to postmortem diffusion of digoxin into the vitreous body. Our results indicate that measurement of digoxin levels in the choroid-retina can aid the postmortem diagnosis of lethal digoxin intoxication.

Key words: Digoxin poisoning – Postmortem diagnosis – Ocular tissues

Zusammenfassung. Nachdem von anderen Autoren tierexperimentell hohe Digitalisglykosidkonzentrationen in okulären Geweben nachgewiesen werden konnten, sollte die Frage geklärt werden, ob durch Bestimmung der Digoxinspiegel in Augengeweben ein Beitrag zur Verbesserung der postmortalen Diagnostik von tödlichen Digoxinintoxikationen geleistet werden kann. Bei mit Digoxin behandelten, in Kliniken verstorbenen Patienten (therapeutisches Kollektiv) sowie in einem Fall einer suicidalen Vergiftung wurden Digoxinkonzentrationen in Glaskörperflüssigkeit und Choroidretina bestimmt. Die in den okulären Geweben bestimmten Werte wurden den Digoxinspiegeln in Femoralvenenblut, Myocard, Niere und Leber gegenübergestellt und unter Berücksichtigung anamnestischer Daten interpretiert. In der Choroidretina wurden im therapeutischen Kollektiv Digoxinkonzentrationen gefunden, die im Mittel deutlich über den in den übrigen Organen bestimmten Werten lagen. Die Streuung der Choroidretinakonzentrationen nach therapeutischer Dosierung war mit der Streuung der übrigen Gewebespiegel vergleichbar. In dem Intoxikationsfall wurde eine ausgesprochen hohe Choroidretinakonzentration festgestellt. Im Vergleich zu den Choroidretinawerten waren die Glaskörperflüssigkeitsspiegel in allen Fällen sehr niedrig; mit einer wesentlichen Verfälschung der Choroidretinakonzentrationen durch eine mögliche Diffusion des Digoxins in den Glaskörper ist danach nicht zu rechnen. Nach unseren Untersuchungsergebnissen ist die Bestimmung des Digoxinspiegels in der Choroidretina in fraglichen Vergiftungsfällen sinnvoll.

Schlüsselwörter: Digoxinintoxikation – Postmortale Diagnostik – Okuläre Gewebe

Introduction

Most instances of lethal digitalis-glycoside intoxication encountered in forensic medical autopsy material involve suicidal or accidental poisonings [1, 9, 17, 18, 29, 37, 40, 45]. However, the extremely narrow therapeutic range of digitalis-glycosides often leads to iatrogenic poisoning. According to Habermann and Löffler [23], digitalis-glycoside intoxication is the most common type of iatrogenic poisoning. Even in hospitalized patients, who can be supervised continually, the incidence of digitalis-glycoside poisoning is reported to be 8%–20% (!); in 3%–21% of cardiac glycoside poisoning cases death was

* This study contains results from the dissertation of P. Harding

Correspondence to: S. Ritz

found to occur "in direct connection with glycoside intoxication" [32].

When lethal iatrogenic poisoning by digitalis-glycosides occurs, charges [of malpractice] may be brought against the attending physician. If legal proceedings are held, it is of utmost importance to determine whether digitalis-glycoside intoxication was in fact the cause of death.

Definitive *postmortem* diagnosis of lethal digitalis-glycoside poisoning is difficult for the following reasons:

- Reliable anamnestic data are often unavailable to the forensic practitioner.
- There are no characteristic morphological findings in cases of lethal digitalis-glycoside poisoning.
- The interpretation of postmortem blood levels is difficult. The following factors in particular must be considered: a) a relatively large overlap exists between therapeutic and toxic digitalis-glycoside levels [8, 32, 39]; b) misleadingly high blood levels can be found before completion of the distribution phase [8, 31, 44]; c) serum digoxin levels can rise before and after death [1, 3–6, 21, 31, 35, 37, 41, 46, 47]; and d) postmortem digoxin blood levels vary according to the site from which the blood is taken [4, 28].
- Tissue levels of digitalis-glycosides, especially digoxin, have been shown to vary widely following therapeutic doses [1–3, 11, 13, 15, 25, 29, 30, 36, 38, 47].

Because of the uncertainty in interpreting postmortem blood levels, digitalis-glycoside concentrations should also be determined in other body fluids and tissues in cases of suspected cardiac glycoside poisoning [3, 5, 6, 9, 24].

However, the wide variation in digitalis-glycoside tissue levels makes it necessary to set very high threshold values for individual organs to enable reliable differentiation between therapeutic cases and cases of intoxication. Accordingly, Aderjan and Rietbrock [5] set threshold values of 400 ng/g for cardiac tissue, 500 ng/g for kidney tissue, and 250 ng/g for liver tissue. These threshold values are reported to be valid for both digoxin and digitoxin [2]. However, the literature reports cases of lethal intoxication in which tissue concentrations clearly fall below these threshold values [9, 29, 40]. According to Härdle and Aderjan [24], such cases can be correctly classified by applying discriminant analysis, which evaluates several parameters simultaneously. It follows that the greater the number of appropriate tissues and body fluids (heart, kidney, liver, femoral vein blood) in which digitalis-glycoside levels can be measured, the more accurately a distinction can be made between therapeutic and toxic cases [24].

Since the reliability of the differentiation between "intoxication" and "non-intoxication" increases with the number of parameters considered, we investigated whether the measurement of digitalis-glycoside levels in ocular tissues could contribute to postmortem diagnosis of lethal poisoning.

Many of the ocular symptoms associated with digitalis-glycoside intoxication (described as early as 1785 by Withering [48]) are apparently not due to an attack by digitalis-glycoside on the central nervous system, but rather to impairment of *retinal* function [10, 16, 19, 20, 22, 26, 33, 34, 43]. The effects of digitalis-glycosides on the eye have even been observed following subtoxic or therapeutic doses [19, 26]. Like the effects of digitalis-glycosides on the heart, they appear to be caused by an inhibition of the Na-K-ATPase [19, 43], which is present in high levels in the retina [12, 19, 43]. Animal experiments have shown that large concentrations of digoxin and digitoxin can be found in the retina and other ocular tissues following administration [10, 19, 20, 27, 33, 34]. Duncker and Herzig [20] found rapid accumulation of digoxin and digitoxin in the retina of guinea pigs to levels at or even above those in the myocardium. The same authors also detected high levels of digoxin and digitoxin in other ocular tissues well supplied with blood, such as the choroid and the iris, whereas low levels were found in ocular tissues poorly supplied with blood, such as the cornea, lens, vitreous body and sclera.

We determined digitalis-glycoside levels in ocular tissues of hospitalized patients who had received therapeutic doses and in cases of suicidal digoxin and digitoxin poisoning. The levels in ocular tissues were compared with those in femoral vein blood, myocardium, kidney and liver, and evaluated in light of the medical history of each patient. The results of our measurements of digoxin levels are reported in this paper; a second study describes our findings for digitoxin [42].

Patients and methods

Postmortem digoxin levels in vitreous humor, choroid-retina[1], serum, myocardium, kidney and liver were measured in 12 patients who had received digoxin therapy (therapeutic group) and in a single case of suicide by β-acetyldigoxin poisoning.

All patients in the *therapeutic group* died in the University Hospital of Christian-Albrechts-University in Kiel; autopsies were performed in the University Institute of Pathology. The postmortem interval (the time between death and autopsy) ranged from 20.5 to 80 h.

Medical records and autopsy protocols were evaluated, and according to the data, all patients had died of natural causes; in no case was a lethal digitalis-glycoside intoxication suspected. Six of the patients were women and 6 men; their ages ranged between 60 and 90 years. In 5 patients, impaired kidney function was present for an extended period prior to death.

At least 7 patients in the therapeutic collective had received *long-term therapy* with digoxin or β-acetyldigoxin (0.25 mg/day digoxin peroral in one case; 0.2 mg/day β-acetyl-digoxin peroral in 5 cases, 0.1 mg/day β-acetyl-digoxin peroral in one case) up to the time of death. In one patient therapy (0.3 mg/day β-acetyldigoxin peroral) had been terminated 10 days prior to death; in 2 other patients it was impossible to determine up to what time the documented therapy (0.25 mg/day digoxin intravenously, 0.2 mg/day β-methyldigoxin peroral) had been carried out. Two further patients died at the onset of therapy, before intravenous β-acetyldigoxin or digoxin saturation could be completed. The interval between the last administration of digoxin and death (therapy-free interval) ranged from 3.5 to 240 h in the therapeutic group.

The *case of suicidal poisoning* involved a 78-year-old woman found dead in her apartment. Three empty 100 tablet containers of "Novodigal" (β-acetyldigoxin, 0.2 mg) and a suicide note were

[1] Choroid and retina were investigated together as "choroid-retina"

9-31

found near the body. No signs of violence were noted at autopsy. Neither macroscopical nor histological findings could explain the cause of death. A substance that could have been the remnants of tablets was found in the intestinal tract extending as far as the ileum.

The specimens we investigated were all obtained at autopsy. The *choroid-retina* and *vitreous humor* were obtained by opening the orbital roof and exposing the bulbus oculi. The wall of the bulbus was opened and the vitreous humor was carefully (to avoid contamination) extracted. The entire posterior wall of the bulbus oculi, including the choroid and retina, was dissected. Since post-mortem preparation of choroid and retina is difficult, both tissues were carefully separated from the sclera and investigated jointly as "choroid-retina". Approximately 100 mg of tissue (wet weight) were thus obtained in each case.

The *myocardium* specimens were taken from the posterior wall of the left ventricle; macroscopically visible subepicardial fat and fibrotic tissues were removed. The *renal* tissue samples included approximately equal portions of cortex and pulp. *Liver* specimens were taken from the center of the right liver lobe. *Blood* was extracted from the femoral vein. Blood samples were centrifuged in order to obtain serum. All samples were stored deep frozen.

Tissue samples were lyophilized, pulverized and homogenized in 0.1 *M* phosphate buffer (pH 7.6). Digoxin was extracted in 2 steps using dichloromethane. All further steps and measurements of serum and vitreous humoral levels were done according to the manufacturer's instructions for the measuring system.

Digoxin levels were measured by fluorescence polarization immunoassay (FPIA; TDx Measuring System for Therapeutica, TDx Digoxin II, Abbott Laboratories).

Table 1. Range of variation, mean values and standard deviations (Mv ± s) for digoxin levels in tissues and body fluids of the entire therapeutic group ($n = 12$) and of the subgroup of seven patients who received long-term therapy

Tissues and body fluids	Digoxin levels (ng/g wet weight or ng/ml)	
	Entire therapeutic group ($n = 12$)	Long-term therapy subgroup ($n = 7$)
Myocardium	45.7–276.1 ng/g ($n = 12$) Mv ± s: 151.1 ± 51.2 ng/g	103.3–275.1 ng/g ($n = 7$) Mv ± s: 160.3 ± 60.0 ng/g
Kidney	50.0–393.1 ng/g ($n = 12$) Mv ± s: 129.0 ± 62.3 ng/g	71.0–243.4 ng/g ($n = 7$) Mv ± s: 140.9 ± 65.2 ng/g
Liver	24.5–175.4 ng/g ($n = 12$) Mv ± s: 70.9 ± 19.7 ng/g	33.6–98.5 ng/g ($n = 7$) Mv ± s: 73.0 ± 22.6 ng/g
Serum	1.2–38.9 ng/ml ($n = 6$) Mv ± s: 3.0 ± 1.6 ng/ml	1.2–5.0 ng/ml ($n = 3$) Mv ± s: 2.6 ± 2.1 ng/ml
Choroid retina	63.9–485.0 ng/g ($n = 12$) Mv ± s: 184.3 ± 95.6 ng/g	140.0–369.9 ng/g ($n = 7$) Mv ± s: 233.1 ± 75.4 ng/g
Vitreous humor	2.2–7.1 ng/ml ($n = 12$) Mv ± s: 3.4 ± 1.3 ng/ml	2.2–6.1 ng/ml ($n = 7$) Mv ± s: 3.8 ± 1.4 ng/ml

Results

1. Therapeutic Group

Table 1 gives an overview of the range of variation in digoxin levels in tissues and body fluids in the therapeutic group as a whole and in the subgroup of 7 patients who had undergone *long-term* digoxin therapy. Digoxin levels in all tissues showed a considerable variation and 4 of the 6 serum digoxin levels exceeded the (clinical) therapeutic range of 0.7–2.2 ng/ml.

Figure 1 is a graphic depiction of the mean digoxin levels and standard deviations in the 7 cases receiving long-term therapy. By far the highest mean value was found in the choroid-retina, followed in descending order by the myocardium, kidney, liver, vitreous humor and serum.

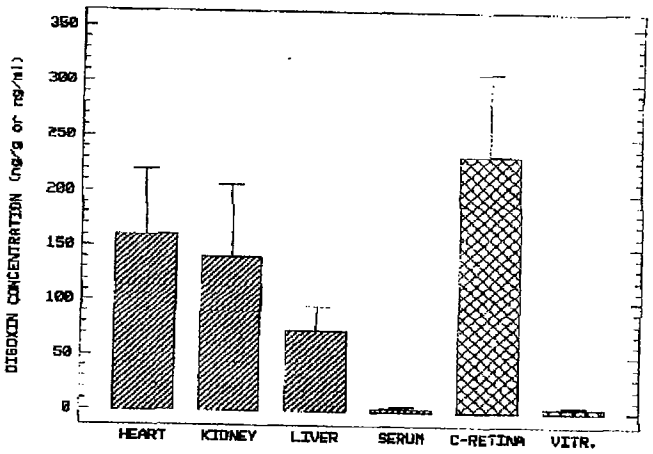


Fig. 1. Mean digoxin levels (columns) in tissues and body fluids of the group of 7 hospital patients who underwent long-term therapy; the respective standard deviations are indicated by the lines on the columns ("C-retina" = Choroid-retina, "Vitr." = vitreous humor)

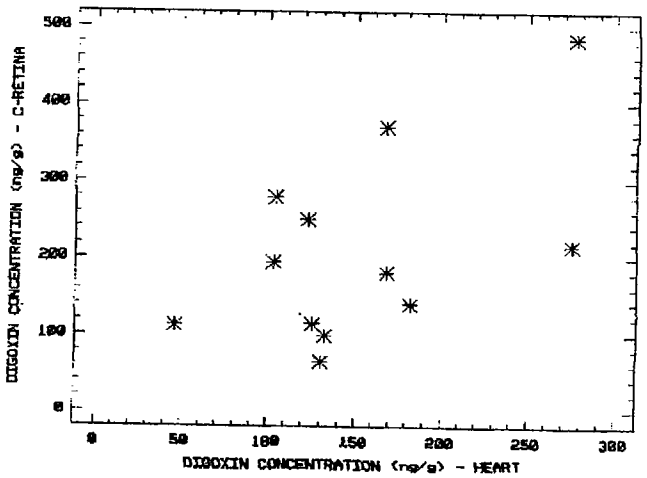


Fig. 2. Digoxin levels in the choroid-retina ("C-retina") of the therapeutic group ($n = 12$) in relation to levels in the myocardium

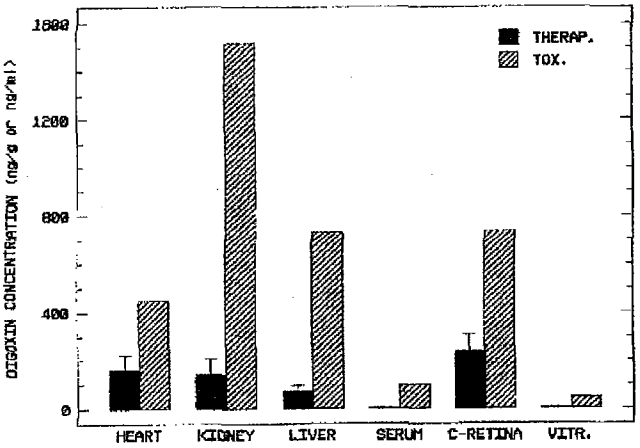


Fig. 3. Comparison of digoxin levels in the case of lethal intoxication (hatched columns) with the mean values for the 7 hospital patients who underwent long-term therapy (dark columns; the lines on the columns indicate standard deviations): "C-retina" = choroid-retina, "Vitr." = vitreous humor

In Figure 2 the digoxin levels in the choroid-retina are compared with those in the "target tissue", the myocardium. To the extent that one can generalize from such a small number of cases, a loose correlation at most exists between digoxin levels in the choroid-retina and those in the myocardium following therapeutic doses. A similar correlation was seen between digoxin levels in other tissues and body fluids, especially between levels in the choroid-retina and vitreous humor.

Digoxin levels in vitreous humor were in some cases greater, in other cases less than the corresponding levels in serum. The ratio of vitreous humoral levels to serum digoxin levels showed no discernable correlation with the length of the therapy-free or postmortem intervals.

In the 5 patients with impaired renal function mean digoxin levels in tissues were higher than in the other patients. One patient in particular had the following high digoxin levels:

- serum: 28.9 ng/ml,
- myocardium: 276.1 ng/g,
- kidney: 293.1 ng/g,
- liver: 175.4 ng/g,
- choroid-retina: 485.0 ng/g,
- vitreous humor: 7.1 ng/ml.

This patient, who was 90 years old and weighed only 42 kg, had received peroral treatment with 0.25 mg/day digoxin. The therapy-free interval could not be determined. Approximately 4 weeks before death a nephrectomy was carried out because of a kidney cell carcinoma. Postoperatively, the patient showed initial improvement but then the condition deteriorated. Death occurred under signs of cardiovascular failure. A lengthy antemortem period of high serum creatinine levels had been observed; the daily digoxin dose was not reduced. Autopsy revealed pre-existing ischemic damage to the heart and signs of cardiovascular failure.

2. *Suicidal Intoxication*

Extremely high digoxin levels were found in the case of suicidal β-acetyldigoxin poisoning:

- serum: 98.4 ng/ml,
- myocardium: 446.9 ng/g,
- kidney: 1514.4 ng/g,
- liver: 727.2 ng/g,
- choroid-retina: 734.2 ng/g,
- vitreous humor: 47.8 ng/ml.

In Fig. 3 these values are compared with the mean digoxin levels in the subgroup of the 7 patients who had undergone long-term therapy. Digoxin levels in the case of suicidal poisoning were many times higher for all tissues investigated, including choroid-retina and vitreous humor, than the mean values in the 7 long-term therapy patients.

Discussion

The diagnosis of lethal digoxin intoxication should be based on the medical history, if available, and on postmortem digoxin levels in tissues and body fluids. In the individual case, digoxin levels should be evaluated in light of published data on patients who had received therapeutic doses and confirmed cases of digoxin poisoning.

It is difficult to compare the results in the extensive literature on digoxin levels in tissues and body fluids following therapeutic and toxic doses, chiefly because of the variety of methods used for measurements. The most commonly employed method has been radio immunoassay, which – like the FPIA we used – also detects variable quantities of digoxin metabolites. Plum and Daldrup [37] suggested that the results of such measurements are difficult to compare since they are affected by the detected metabolites, which in turn depend on the extraction method used.

Furthermore, the site from which the samples are taken as well as the type of patient population can influence findings on postmortem digoxin levels [1, 4, 7, 28, 30, 47] and thus reduce the validity of comparisons between different studies.

The digoxin levels we measured in myocardium, kidney, liver, and femoral vein blood agree well with levels reported by many authors [1, 3, 5, 7, 29, 30]. They differ, however, from the results of others, for example Ottoson et al. [36] and Weinmann et al. [47]. Ultimately, the widely divergent findings on digoxin levels following therapeutic doses and in cases of digoxin poisoning are "only relatively and not directly comparable with each other" [5].

Distinguishing between "intoxication" and "non-intoxication" is complicated even more by the fact that therapeutic doses of digoxin produce widely varied concentrations in body fluids and tissues [1, 11, 13, 15, 25, 29, 30, 36, 38, 47], which makes determination of the therapeutic range difficult.

In our therapeutic group also large variations in the digoxin levels in body fluids and tissues were found (Table 1). Even the myocardium, the "target organ" of digitalis-glycosides, showed widely divergent digoxin levels following therapeutic doses. This has been explained by fluctuations in the digitalis-glycoside levels due to pathological, structural and metabolic changes [15, 47] in the tissue. Moreover, a variable, nonspecific, receptor-independent binding of digitalis-glycosides could also play a role [3, 5, 11, 13].

Some authors have recommended measuring digoxin levels in *vitreous humor* in cases of suspected poisoning [14, 18, 35, 46].

Di Maio et al. [18] suggested that lower levels in vitreous humor than in blood indicate a time of death prior to completion of the distribution phase. Our findings could not confirm this; the ratio of vitreous humoral levels to serum levels showed no discernable relationship to the length of time between the last digoxin intake and death.

Margot et al. [35] regarded digoxin levels exceeding 6 ng/ml in vitreous humor to be an indication of lethal intoxication. In several patients in our therapeutic group vitreous humoral levels of approximately 6 ng/ml were found. A postmortem diffusion of digoxin from the retina into the vitreous body may explain these high values. Binnion and Frazer [10] reported such a postmortem diffusion in animals. However, no reliable correlation was found between the vitreous humoral levels, the choroid-retinal levels and the length of the postmortem interval. It appears that the extent of postmortem diffusion of digoxin into the vitreous body can vary widely.

In the *choroid-retina* we found high postmortem digoxin levels after therapeutic doses; the mean value was clearly above those in other tissues (Fig. 1). This agrees with animal studies showing that the blood-retina barrier (in contrast to the blood-brain barrier) appears to be extremely porous to digoxin, and that an enhancement of the digoxin concentration is especially evident in the retina [10, 19, 20, 27, 33, 34].

In the therapeutic group, the variation in digoxin levels in the choroid-retina was comparable to that in the other tissues examined.

It appears that at best a loose correlation exists between digoxin levels in the choroid-retina and those in the myocardium (Fig. 2) and in other tissues. This may be due to alterations in digoxin levels caused by pathological changes in structure and function of the tissues [15, 19, 47] and to a variably high rate of nonspecific binding of digoxin in tissues, as has been described for the myocardium [11, 13, 32, 47].

Compared to choroid-retinal levels, digoxin levels in the vitreous humor were low. Hence, significant distortion of choroid-retinal levels due to postmortem diffusion of digoxin into the vitreous body is unlikely.

In the single case of suicidal intoxication, the diagnosis of lethal digoxin intoxication was easily made on the basis of the massive digoxin levels in all body fluids and tissues.

An example of a diagnostically difficult case is the one patient in the therapeutic group where digoxin levels far exceeded the mean levels in all tissues (myocardium: 276.1 ng/g; kidney: 293.1 ng/g; liver 175.4 ng/g; choroid-retina: 485.0 ng/g). The serum level in particular was remarkably high (28,9 ng/ml) even in the light of a possible rise in serum levels before or after death. The levels in the myocardium, kidney, and liver were below the threshold values suggested by Aderjan and Rietbrock [5]. On the other hand, the concentrations in these tissues exceeded those reported by some authors in cases of lethal intoxication [9, 29, 40]. In such critical cases the clinical data – if available – must be considered. In our patient (a 90-year-old male weighing only 42 kg) impairment of renal function – the most frequent contributing cause of digoxin intoxication [32] – began long before death; the daily digoxin dose was not reduced. The high postmortem digoxin levels found in body fluids and tissues support the hypothesis that impaired renal function resulted in an accumulation of digoxin from therapeutically administered doses that were too high under the circumstances. The patient's general condition before death was poor. "Typical" symptoms of digitalis-glycoside intoxication, in particular cardiac arrythmia, were not mentioned in the medical records; however, electrocardiogram tests were not made in the last days antemortem. Hence, the clinical data could neither confirm nor exclude (lethal) digoxin intoxication.

In this and in similar cases in which digoxin has not been ingested in excessive amounts and where suspicion of lethal digoxin intoxication is neither supported nor ruled out by anamnestic data, it is imperative that levels are measured in as many appropriate tissues and body fluids as possible [5, 24, 40].

Our results indicate that measurement of digoxin levels in the choroid-retina could contribute to improving postmortem diagnosis of digoxin intoxication. However, before choroid-retinal levels can be employed in cases of suspected poisoning, studies of sufficiently large series of therapeutic and toxic cases must provide reliable data for comparison.

References

1. Aderjan R (1980) Probleme bei der Beurteilung von Digoxinvergiftungen. Beitr Gerichtl Med 38:223–226
2. Aderjan R (1983) Digitoxin-Konzentrationen in postmortal entnommenen Blut- und Gewebeproben des Menschen nach therapeutischer Dosierung. In: Gillmann H, Storstein L (eds) Digitalistherapie heute. Verlag für angewandte Wissenschaften, München, pp 49–57
3. Aderjan R (1985) Herzglykosid-Intoxikationen; Postmortale Befundung bei Digoxin und Digitoxin. Zentralbl Rechtsmed 27:379–390
4. Aderjan R, Mattern R (1980) Zur Wertigkeit postmortaler Digoxin-Konzentrationen im Blut. Z Rechtsmed 86:13–20
5. Aderjan R, Rietbrock N (1983) Stellenwert von Konzentrationsmessungen zur postmortalen Klärung von Herzglykosid-Intoxikationen. In: Rietbrock N, Schnieders B, Schuster B (eds) Wandlungen in der Therapie der Herzinsuffizienz. Vieweg, Braunschweig Wiesbaden, pp 221–236
6. Aderjan R, Buhr H, Schmidt G (1979) Investigation of cardiac glycoside levels in human post mortem blood and tissues determined by a special radioimmunoassay procedure. Arch Toxicol 42:107–114

9-34

7. Andersson K-E, Bertler A, Wettrell G (1975) Postmortem distribution and tissue concentrations of digoxin in infants and adults. Acta Paediatr Scand 64:497–504
8. Anschütz F (1984) Möglichkeiten und Grenzen der Digitalisspiegelmessung. Ärztl Lab 30:377–381
9. Arnold W, Püschel K (1979) Toxikologische und morphologische Befunde bei Digoxinvergiftung in forensischer Sicht. Z Rechtsmed 83:265–272
10. Binnion PF, Frazer G (1980) (^{3}H)Digoxin in the optic tract in digoxin intoxication. J Cardiovasc Pharmacol 2:699–706
11. Binnion PF, Morgan JM, Stevenson HM, Fletcher M (1969) Plasma and myocardial digoxin concentrations in patients on oral therapy. Br Heart J 31:636–640
12. Bonting SL, Caravaggio LL, Canandy MR (1964) Studies on sodium-potassium-activated adenosine triphosphatase. X. Occurrence in retinal rods and relation to rhodopsin. Exp Eye Res 3:47–56
13. Carroll PR, Gelbart A, O'Rourke MF, Shortus J (1973) Digoxin concentrations in the serum and myocardium of digitalised patients. Aust N Z J Med 3:400–403
14. Coe JI (1981) Forensic aspects of cardiac medications. Am J Forensic Med Pathol 2:329–332
15. Coltart J (1978) Significance of plasma concentration of digoxin in relation to the myocardial concentration of the drug. In: Bodem G, Dengler HJ (eds) Cardiac glycosides. International Boehringer Mannheim Symposia. Springer, Berlin Heidelberg New York, pp 135–158
16. Denden A (1962) Elektroretinographische Spektralsensitivität bei Farbigsehen nach Acetyldigoxinbehandlung. Graefes Arch Clin Exp Ophthalmol 165:185–194
17. Dickson SJ, Blazey ND (1977) Postmortem digoxin levels – two unusual case reportes. Forensic Sci 9:145–150
18. DiMaio VJM, Garriott JC, Putnam R (1975) Digoxin concentrations in postmortem specimens after overdose and therapeutic use. J Forensic Sci 20:340–347
19. Duncker G (1990) Untersuchungen zur Wirkung von Digitalisglykosiden am Auge. Habilitationsschrift, Kiel
20. Duncker G, Herzig S (1989) On the ocular distribution of cardiac glycosides in guinea pigs following acute administration. Graefes Arch Clin Exp Ophthalmol 227:55–59
21. Eriksson M, Lindquist O, Edlund B (1984) Serum levels of digoxin in sudden cardiac deaths. Z Rechtsmed 93:29–32
22. Gibson HC, Smith DM, Alpern M (1965) II_5 specificity in digitoxin toxicity. Arch Ophthalmol 74:154–158
23. Habermann E, Löffler H (1983) Spezielle Pharmakologie und Arzneitherapie. 4. Aufl, Springer, Berlin Heidelberg New York Tokyo
24. Härdle W, Aderjan R (1983) Klassifizierung von Digoxin-Blut- und Gewebekonzentrationen bei Vergiftungsverdacht. Z Rechtsmed 91:1–15
25. Härtel G, Kyllönen K, Merikallio E, Ojala K, Manninen V, Reissell P (1976) Human serum and myocardium digoxin. Clin Pharmacol Ther 19:153–157
26. Haustein K-O, Olumanns G, Rietbrock N, Alken RG (1982) Differences in color vision impairment caused by digoxin, digitoxin, or pengitoxin. J Cardiovasc Pharmacol 4:536–541
27. Hollwich F, Seifert J (1990) Verteilung von radioaktiv markiertem Digitalis am Auge. Klin Monatsbl Augenheilk 196:158–159
28. Holt DW, Benstead JG (1975) Postmortem assay of digoxin by radioimmunoassay. J Clin Pathol 28:483–486
29. Iisalo E, Nuutila M (1973) Myocardial digoxin concentrations in fatal intoxications. Lancet 1:257
30. Karjalainen J, Ojala K, Reissell P (1974) Tissue concentrations of digoxin in an autopsy material. Acta Pharmacol Toxicol 34:385–390
31. Koren G, Parker R (1985) Interpretation of excessive serum concentrations of digoxin in children. Am J Cardiol 55:1210–1214
32. Larbig D, Haasis R, Kochsiek K (1978) Die Glykosidkonzentration und ihre klinische Bedeutung. Forum Cardiologicum 15, Studienreihe Boehringer Mannheim: 77–105
33. Lissner W, Greenlee JE, Cameron JD, Goren SB (1971) Localization of tritiated digoxin in the rat eye. Am J Ophthalmol 72:608–614
34. Lufkin MW, Harrison CF, Henderson JW, Ogle KN (1967) Ocular distribution of digoxin-^{3}H in the cat. Am J Ophthalmol 64:1134–1140
35. Margot PA, Finkle BS, Peat MA (1983) Analysis and problems of interpretation of digoxin in post-mortem blood and tissues. Proc West Pharmacol Soc 26:393–396
36. Ottoson A, Edvinsson L, Sjögren A, Löwenhielm P (1988) Digoxin, magnesium, and potassium levels in a forensic autopsy material of sudden death from ischemic heart disease. Z Rechtsmed 101:27–36
37. Plum J, Daldrup T (1985) Verteilung von Digoxin, Digitoxin und ihren kardioaktiven Metaboliten im menschlichen Herz- und Nierengewebe. Eine post mortem – Untersuchung. Z Rechtsmed 94:257–272
38. Redfors A, Bertler A, Schüller H (1973) The ratio between myocardial and plasma levels of digoxin in man. In: Storstein O (ed) Symposium on digitalis. Gyldendahl Norsk, Oslo, pp 265–269
39. Rietbrock N, Oeff F, Maertin K, Kuhlmann J (1978a) Glykosidkonzentrationen im Plasma und Intoxikationshäufigkeit nach β-Methyldigoxin und β-Acetyldigoxin unter standardisierten Bedingungen. Eine prospektive Studie. Herz Kreisl 10:267–273
40. Rietbrock N, Wojahn H, Weinmann J, Hasford J, Kuhlmann J (1978b) Tödlich verlaufene β-Methyldigoxin-Intoxikation in suicidaler Absicht. Dtsch Med Wochenschr 103:1841–1844
41. Ritz S, Kaatsch H-J (1990) Zur Interpretation von postmortalen Digoxinspiegeln: Überprüfung eines "Korrekturfaktors" für postmortal gemessene Digoxinkonzentrationen im Blut. Z Rechtsmed 103:573–580
42. Ritz S, Harding P, Martz W, Schütz HW, Kaatsch H-J (1992) Measurement of digitalis-glycoside levels in ocular tissues: a way to improve postmortem diagnosis of lethal digitalis-glycoside poisoning? II. Digitoxin. Int J Leg Med (in press)
43. Robertson DM, Hollenhorst RW, Callahan JA (1966) Receptor function in digitalis therapy. Arch Ophthalmol 76:852–857
44. Rosenkranz B (1986) Kosten-Nutzen-Analyse der Plasmakonzentrationsbestimmung von Digoxin. In: Frölich JC (ed) Plasmaspiegel – Wirkungsbeziehungen von Pharmaka. Fischer, Stuttgart New York, pp 155–166
45. Steentoft A (1973) Fatal digitalis poisoning. Acta Pharmacol Toxicol 32:353–357
46. Volpahl TE, Coe JI (1978) Correlation of antemortem and postmortem digoxin levels. J Forensic Sci 23:329–334
47. Weinmann J, Hasford J, Kuhlmann J, Bippus PH, Lichey J, Rietbrock N (1979) Digoxinkonzentrationen in Plasma und Gewebe. Med Klin 74:613–619
48. Withering W (1785) An account of the foxglove and some of its medical uses: with practical remarks on dropsy, and other diseases. M Swinney, Birmingham

Forensic Science, 6 (1975) 31—39
© Elsevier Sequoia S.A., Lausanne — Printed in The Netherlands

COMPARATIVE TOXICOLOGY IN VITREOUS HUMOR AND BLOOD

WILLIAM Q. STURNER* and JAMES C. GARRIOTT

Southwestern Institute of Forensic Sciences and Department of Pathology, University of Texas Southwestern Medical School, Dallas, Texas 75235 (U.S.A.)

SUMMARY

Drug and toxic substances were detected in blood and vitreous humor in fifty-six cases, in which causes of death were both from an overdose of the particular substances and from other unrelated causes. Five instances are reported in which two drug substances were detected in blood and vitreous humor from the same subject. Patients having long survival times, as well as those dying from unrelated causes, reveal drug values to approach unity, when the blood and vitreous concentrations are compared. The ratios reached at equilibrium probably depend on solubility of the drug in vitreous humor, lipid solubility and the percentage protein-bound in the blood. The vitreous humor provides another parameter of testing and may be useful in studies of survival time.

INTRODUCTION

The first publication of the use of vitreous humor for toxicologic analysis involved ethyl alcohol determination and compared values with blood specimens obtained simultaneously at autopsy [1]. Subsequent reports have confirmed the usefulness and accuracy of this approach [2—4]. More recently, investigators have reported on the determination of drugs in vitreous humor and their relationship to concentrations in blood and other tissues [5—9]. The substances described have been barbiturates, meprobamate, salicylates, ethchlorvynol, digoxin, quinine and lithium. Our experience covering the past three years has included a variety of pharmaceutical agents and toxic substances. A presentation of these findings in fifty-six cases comprises the present study.

METHODS

Standard procedures for withdrawing blood from the heart and preserving the specimen in chemically clean vacutainer tubes were employed. Vitreous

*Present address: Office of the State Medical Examiner, Department of Health, 75 Davis Street, Providence, Rhode Island 02908 (U.S.A.).

TABLE 1

Drug levels in blood and vitreous humor

Single drug analysis

Case	A-R-S	Substance	Blood (μg/ml)	Vitreous (μg/ml)	Vitreous blood Ratio	Alcohol (% w/v)	Survival time (h)	Cause of death*	Manner of death
1	17 w/f	Propoxyphene	9.20	0.50	0.05		<5	OD	Suicide
2	43 w/f	Propoxyphene	9.00	0.60	0.07	0.02	UNK	OD	Suicide
3	38 w/f	Propoxyphene	8.10	0.80	0.10	0.20	9	OD	Undetermined
4	18 w/f	Propoxyphene	13.90	2.40	0.17		<8½	OD	Suicide
5	24 n/f	Propoxyphene	8.70	1.63	0.19		<8	OD	Suicide
6	57 w/f	Propoxyphene	3.00	0.67	0.22		<10	OD	Suicide
7	50 w/f	Propoxyphene	4.75	1.05	0.22		<8	OD	Suicide
8	16 n/f	Propoxyphene	2.05	0.60	0.29	0.20	<9½	OD	Undetermined
9	25 w/f	Propoxyphene	5.50	1.80	0.33		>1½, <8	OD	Suicide
10	31 n/m	Propoxyphene	8.90	3.00	0.34		<15	OD	Suicide
11	63 w/m	Secobarbital	25.00	5.00	0.20	0.17	UNK	OD Secobarbital	Suicide
12	20 w/m	Secobarbital	21.00	4.90	0.23	0.01	UNK	OD Secobarbital and diazepam	Suicide
13	21 w/m	Secobarbital	3.70	1.50	0.40		UNK	GSW	Suicide
14	27 w/m	Secobarbital	5.00	5.00	1.00		UNK	CO Poisoning and smoke inhalation	Accidental
15	27 w/f	Amitriptyline	40.00	0.80	0.02	0.01	<3	OD	Suicide
16	53 w/f	Amitriptyline	17.00	2.20	0.13		>10	OD	Suicide
17	23 w/f	Amitriptyline	5.85	0.90	0.15	0.16	>15	OD Diazepam and amitriptyline	Suicide
18	87 w/f	Digoxin	38.60**	2.80**	0.07		UNK	OD Digoxin	Suicide
19	33 n/f	Digoxin	3.10	1.20	0.39		UNK	ASHD	Natural
20	72 n/f	Digoxin	4.00 (hemolyzed)	1.60	0.40		UNK	ASCVD	Natural
21	59 w/f	Digoxin	4.70	2.10	0.45		UNK	ASHD	Natural

PLAINTIFFS' EXHIBITS 012619

Case 2:08-md-01968 Document 580-27 Filed 09/08/11 Page 26 of 64 PageID #: 24501

TABLE 1 (continued)

Case	A-R-S	Substance	Blood (μg/ml)	Vitreous (μg/ml)	Vitreous/ blood Ratio	Alcohol (% w/v)	Survival time (h)	Cause of death	Manner of death
38	44 w/m	Ethchlorvynol	28.30	13.00	0.46	0.08	<20	OD propoxyphene and ethchlorvynol	Suicide
39	76 w/m	Phenobarbital	6.20	2.90	0.47		UNK	GSW's thorax	Suicide
40	67 w/f	Oxycodone	1.30	0.50	0.38		<24	OD Oxycodone and diazepam	Suicide
41	29 n/m	Propylhexedrine	1.80	1.70	0.95		UNK	IV narcotism; cor pulmonale	Unclassified
42	35 n/m	Paraldehyde	800.0	1040.0	1.30		13 (hospital)	OD	Accidental
43	28 w/f	Cocaine	8.5	3.8	0.45		UNK	OD	Undetermined
44	30 w/m	Meprobamate	54.00	25.00	0.46		UNK	OD meprobamate and propoxyphene	Undetermined
45	49 w/f	Methapyrilene	9.00	1.20	0.13		UNK	OD Sominex^{R}; fatty liver	Suicide
46	65 w/f	Methyprylon	47.80	58.40	1.22		UNK	ASCVD; (Ca mouth)	Natural
47	39 w/m	Cyanide	100.00	10.00	0.10		UNK	OD	Suicide
48	67 w/f	Insulin	488.3 mU/ml	14.5 mU/ml	0.03		UNK	ASCVD Diabetes mellitus	Natural

* OD = overdose, GSW = gunshot wound, ASHD = arteriosclerotic heart disease. ** All digoxin values expressed as ng/ml.

TABLE II

Drug levels in blood and vitreous humor

Multiple drug analyses

PLAINTIFFS' EXHIBITS 012620

9-38

Case 2:08-md-01968 Document 580-27 Filed 09/08/11 Page 27 of 64 PageID #: 24502

										tyline		Suicide
							UNK			OD		
18	87 w/f	Digoxin	38.60**	2.80**	0.07					Digoxin		
							UNK			ASHD		Natural
19	33 n/f	Digoxin	3.10	1.20	0.39							
							UNK	ASCVD				Natural
20	72 n/f	Digoxin	4.00 (hemolyzed)	1.60	0.40							
			4.70	2.10	0.45		UNK	ASHD				Natural
21	59 w/f	Digoxin	(hemolyzed)									
			12.10	8.50	0.70		UNK	OD				Accidental
22	88 w/f	Digoxin						Digoxin				
			1.10	0.80	0.73		UNK	ASCVD				Suicide
23	80 n/m	Digoxin	4.70	3.90	0.83		UNK	ASHD				Natural
24	52 w/f	Digoxin	1.50	1.80	1.20		UNK	ASHD				Natural
25	55 w/f	Digoxin	14.70	2.60	0.18		UNK	OD				Suicide
26	20 w/f	Pentazocine	4.60	0.97	0.21	0.17	UNK	OD Pen-				Undetermined
27	58 w/f	Pentazocine						tazocine				
								chloral				
								hydrate				
						0.10	<12	OD				Suicide
28	41 w/f	Pentazocine	3.80	0.90	0.24		<7	OD				Suicide
29	22 w/v	Amphetamine	6.67	0.50	0.07			Ampheta-				
								mine and				
								amitriptyline				
							UNK	Undeter-				Undetermined
								mined				
30	23 w/m	Amphetamine	0.16	0.06	0.47				>3 h, <18	OD		Unclassified
			0.70	0.10	0.14	<0.01	UNK	Acute IV				Unclassified
31	20 w/m	Methadone	0.60	0.10	<0.16			narcotism				
32	28 n/f	Methadone					<14	OD				Suicide
			0.70	0.20	0.29			Imipramine				
33	25 w/m	Imipramine	10.50	1.90	0.18							
		Desipramine	4.70	1.40	0.30	0.09	UNK	OD				Suicide
34	30 w/m	Imipramine	1.60	0.10	<0.06			Imipramine				
		Desipramine	844.00	467.00	0.55		10	OD				Suicide
35	53 w/f	Salicyclic acid	6.10	1.00	0.16		UNK	ASHD				Natural
36	65 w/m	Quinidine	1.88	0.72	0.38		<5	OD				Suicide
37	46 w/f	Codeine						barbiturate,				
								codeine, di-				
								azepam,				
								hydroxyzine,				
								acetamino-				
								phen				

(continued)

* OD = overdose, GSW = gunshot wound, ASHD = arteriosclerotic heart disease. ** All digoxin values expressed as ng/ml.

TABLE II

Drug levels in blood and vitreous humor

Multiple drug analyses

Case	A-R-S	Substance	Blood (μg/ml)	Vitreous (μg/ml)	Vitreous/blood ratio	Survival time (h)	Cause of death	Manner of death
49	69 w/m	Propoxyphene Methaqualone	0.50 0.70	0.10 0.20	0.20 0.29	<2	OD Methaqualone, propoxyphene, pentobarbital and diazepam	Suicide
50	21 w/f	Amitriptyline Diazepam	1.88 0.50	0.20 5.10	0.11 10.20	72 (hospital)	OD Amitriptyline, diazepam	Suicide
51	58 w/f	Pentazocine Lidocaine	0.14 0.94	0.40 0.76	2.88 0.81	3 weeks (hospital)	Complications of GSW	Undetermined
52	32 w/f	Diazepam Amobarbital and secobarbital	0.30 4.00	0.20 0.02	0.67 <0.01	UNK	OD Diazepam, secobarbital and amobarbital	Suicide
53	41 w/m	Glutethimide Phenobarbital	12.9 11.0	7.7 5.7	0.60 0.52	UNK	OD Glutethimide and phenobarbital	Suicide

PLAINTIFFS' EXHIBITS 012622

P-40

Case 2:08-md-01968 Document 580-27 Filed 09/08/11 Page 29 of 64 PageID #: 24504

humor specimens were simultaneously withdrawn and stored as previously described [1]. Accepted analytical methods for the determination of drugs and other substances in blood and other body fluids were utilized. These included ultra-violet spectrophotometry and gas—liquid chromatography [10], except in assays for digoxin and insulin where radioimmunoassay was employed [11,12]. Cases were included if one or more drugs were recovered, and also when death was by means other than drug or chemical intoxication, but those in which blood or vitreous was negative for the substance analyzed were not included. The results of single drug analyses are listed in Table I. The five instances in which concentrations of two drugs were determined are shown in Table II.

RESULTS

There were ten cases in which propoxyphene was detected in blood and vitreous humor. The ratio of concentrations in vitreous humor to that of blood was relatively consistent, ranging between 0.05 and 0.34. In three instances, ethyl alcohol was detected. In each of the ten cases, the cause of death was due to an overdose of propoxyphene, and in those with low vitreous to blood ratios, death probably ensued before equilibrium between the blood and vitreous humor could be established.

In the five instances of secobarbital detection, the ratios ranged from 0.20 to 1.00. The two highest ratios, namely 0.40 and 1.00, occurred where the cause of death was other than overdose of the drug (see Table I, cases 13 and 14). In the latter case equilibrium had already been established and one presumes that the ingestion of the secobarbital took place long before the incident of death from smoke inhalation and carbon monoxide poisoning. In another case, not included in Table I, an 86 year old woman died of a suicidal overdose of secobarbital. The body had been embalmed prior to analysis, probably resulting in an artificially low blood level. Results were as follows: blood, 0.64 mg/100 ml; vitreous, 0.40 mg/100; vitreous/blood ratio, 0.63.

In the three cases in which amitriptyline was the substance recovered, the ratios varied considerably and the very high blood level noted in case 15 can be ascribed to a massive ingestion with a short survival time (<3 hours) and the subsequent lack of time for tissue distribution and equilibrium to take place. The lower blood levels noted in the other two instances, and consequent higher vitreous ratios, probably resulted from much longer survival time (>15 hours and >10 hours, respectively).

Eight cases in which digoxin was recovered are shown, with two instances consistent with an overdose of the substance, one a known suicide. It has been observed that many cases of death of individuals taking digoxin have toxic levels of this drug in the blood at the time of death [8]. Some of these could be examples of inadvertent overdose. Hemolysis of the blood in postmortem samples interferes with the assay of digoxin by some radioimmuno-

assay tec
vitreous
The bloc
remainde
few hou
proxima
0.7—0.8
of vitreo
the lag i
body me
Three
vitreous/
overdose
therapeu
levels in
There
containi
presence
methane
Two i
the subs
not nece
time is p
ratios ar
The o
stances.
vitreous
paraldeh
Table
the bloo
instance
amount
procedu
were ind

DISCUSSI

The v
drug sul
paramet
survival
It has al
turates i
time of
and mer

assay techniques, resulting in artificially low digoxin values, but levels in the vitreous humor can be used to support elevated blood levels when this occurs. The blood in two of the cases (20 and 21) was severely hemolyzed, while the remainder had no observable hemolysis. At equilibrium, occurring within a few hours after administration of the dose, the vitreous level of digoxin is approximately equivalent to that in the blood, probably having a ratio of 0.7—0.8 due to the 23—33% protein binding of this drug [11]. The finding of vitreous/blood ratios greater than one in some cases may be explained by the lag in re-equilibrating with the blood after that level declines, owing to body metabolism and excretion [8].

Three instances of pentazocine analyses revealed closely approximating vitreous/blood ratios. The two cases of amphetamine detection indicate one overdose value in an instance of suicide (6.67 μg/ml) and one example of a therapeutic level (0.16 μg/ml), neither of those having attained equivalent levels in the vitreous humor.

There were two cases of death resulting from inhalation of Freon-containing aerosols, in which both blood and vitreous humor revealed the presence of Freons 11 and 12 (trichlorofluoromethane and dichlorodifluoromethane, respectively). Quantitation of these gases was not attempted.

Two instances of methadone detection, one diagnosed as an overdose of the substance, are included. Because blood levels are extremely variable and not necessarily indicative of intoxication and when overdose occurs, survival time is prolonged with this drug, it is not surprising that the vitreous/blood ratios are similar in the two instances.

The other instances presented in Table I involved a variety of drug substances. Of interest is the case of methyprylon (case 46), in which the blood/vitreous ratio was greater than one (1.22). This was also noted in the case of paraldehyde intoxication (case 42).

Table II depicts the five instances in which two drugs were recovered in the blood and vitreous humor. It should be pointed out that other similar instances involving multiple drug ingestion are not included here because the amount of vitreous humor was inadequate to perform more than one test procedure. These latter cases were placed in Table I, and other drugs present were indicated herein.

DISCUSSION AND CONCLUSION

The vitreous humor provides a further medium for the evaluation of drug substances in autopsy cases, and the values obtained may be a useful parameter when compared with blood concentrations for the assessment of survival time, or time between administration of the substance and death. It has also been observed that a study of the ratio of concentrations of barbiturates in the liver to those in the blood can also be used to estimate the time of survival after overdose [13]. A previous study analyzing barbiturate and meprobamate concentrations in vitreous humor and blood, concludes

that the substances are distributed by diffusion and that when diffusion equilibrium occurs, the ultra-filtrate blood levels and those in the vitreous humor are identical. These observers found that barbiturates were bound to protein in varied amounts, depending on the case history, as well as the type of barbiturate present [5].

Coe has recently described seventeen cases in which barbiturates, salicylates, ethchlorvynol and meprobamate analyses were performed in blood and vitreous humor [6]. His findings indicated more variation between blood and vitreous humor than those reported by Felby and Olsen, and indicated potential difficulty in separating toxic from therapeutic levels of these drugs, based on vitreous humor levels alone. He also reported an instance of analysis of barbiturate in vitreous humor following embalming, showing a liver value of 9.5 mg% and a vitreous humor level of 0.6 mg%, but was unable to determine the exact quantity in the blood. In the instance mentioned above, embalming had also been performed and the ratio was approximately three times higher than somewhat similar cases of secobarbital overdose also analyzed (see cases 11 and 12). This could be explained by the long survival time allowing close equilibrium with the blood and/or poor recovery from the blood and dilution due to the embalming fixative.

From the data observed here and those of other workers [1,3,5,6,8,9], several generalizations may be made concerning the concentration of drugs in the vitreous humor. The more water-soluble drugs and those least affected by the protein-binding factors in the blood are readily diffusible from the blood into the vitreous humor, providing they have sufficient lipid solubility to penetrate the blood—vitreous barrier. The concentrations in the latter specimen are approximately equivalent to those in blood at the point when equilibrium is reached in the body compartments. In some cases, e.g. paraldehyde, methyprylon and ethanol, high water solubility of the drug may permit a higher concentrations of the drug in the vitreous humor owing to the higher water content. Lipid solubility appears to be the major factor determining the rate of diffusion of weakly acid and basic drugs [9]. Therefore, drugs with low lipid solubility would have a greater lag time in reaching equilibrium, and some may never reach this state during the life of the drug in the plasma. It has also been shown that the concentration of the drug in plasma affects the equilibrium ratio with some drugs, e.g. salicylic acid [9]. As the vitreous humor is acid with respect to the plasma, the weakly alkaline drugs should reach the highest concentrations at equilibrium, and may do so irregardless of extensive protein-binding in the blood [9]. For some drugs affected by protein-binding, the vitreous concentration should equal that of the plasma "ultrafiltrate" or protein free component [5]. A vitreous/blood ratio of greater than one can also be observed with some drugs owing to an apparent lag in equilibration with the blood, such as observed in some cases with digoxin. This would occur in non-fatal cases where the blood level declines due to normal metabolism and excretion, and it has been observed in digoxin-taking patients who died of causes other than overdose [8]. The de-

termina
of the l

REFERI

1 W.Q.
and
2 M.S.
post
3 S. F.
hum
4 J.I.
bloo
5 S. F.
bam
6 J.I.
Sci.
7 J.I.
ture.
8 V.J.I
over
9 P.N.
tal, a
29 (
10 E. F
extr
11 D.C.
trati
12 T.W.
digo
13 C.N.
Bioc
14 A.S.
Toxi

9-43

termination of this ratio can therefore be of forensic utility in the estimation of the length of time between administration of a drug and death.

REFERENCES

1 W.Q. Sturner and R.J. Coumbis, The quantitation of ethyl alcohol in vitreous humor and blood by gas chromatography. Am. J. Clin. Path., 46 (1966) 349—351.
2 M.S. Leahy, E.R. Farber and M.T. Meadows, Quantitation of ethyl alcohol in the postmortem vitreous humor. J. Forens. Sci., 13 (1968) 498—502.
3 S. Felby and J. Olsen, Comparative studies of postmortem ethyl alcohol in vitreous humor, blood and muscle. J. Forens. Sci., 14 (1969) 93—101.
4 J.I. Coe and R.E. Sherman, Comparative study of postmortem vitreous humor and blood alcohol. J. Forens. Sci., 15 (1970) 185—190.
5 S. Felby and J. Olsen, Comparative studies of postmortem barbiturate and meprobamate in vitreous humor, blood and liver. J. Forens. Sci., 14 (1969) 507—514.
6 J.I. Coe, Further thoughts and observations on postmortem chemistries. Forens. Sci. Gaz., 4 (5) (1973) 2—6.
7 J.I. Coe, Postmortem chemistry: practical considerations and a review of the literature. J. Forens. Sci., 19 (1974) 13—32.
8 V.J.M. DiMaio, J.C. Garriott and R. Putnam, Digoxin—an unsuspected source of drug overdose. J. Forens. Sci., (1975) in press.
9 P.N. Sorensen, The penetration of quinine, salicylic acid, PAS, salicyluric acid, barbital, and lithium across the vitreous barrier of the rabbit eye. Acta Pharmacol. Toxicol., 29 (1971) 194—208.
10 E. Foerster and M.F. Mason, Preliminary studies on the use of n—butyl chloride as an extractant in a drug screening procedure. J. Forens. Sci., 19 (1974) 155.
11 D.C. Evered, C. Chapman and C.J. Hayter, Measurement of plasma digoxin concentration by radioimmunoassay. Brit. Med. J., 3 (1970) 427—428.
12 T.W. Smith, V.P. Butler and E. Haber, Determination of therapeutic and toxic serum digoxin concentrations by radioimmunoassay. N. Eng. J. Med., 281 (1969) 1212—1216.
13 C.N. Hale and P.J. Randle, Immunoassay of insulin with antibody precipitate. Biochem. J., 88 (1963) 137.
14 A.S. Curry and I. Sunshine, The liver/blood ratio in cases of barbiturate poisoning. Toxicol. Appl. Pharmacol., 2 (1960) 602—606.

Downloaded from jcp.bmj.com on 6 January 2009

J. clin. Path., 1975, **28**, 483-486

Postmortem assay of digoxin by radioimmunoassay

DAVID W. HOLT AND JOHN G. BENSTEAD

From the Poisons Unit, Guy's Hospital, London SE1 and the Department of Pathology, Southport General Infirmary, Southport, Lancs

SYNOPSIS Analysis of postmortem blood samples from patients previously on maintenance digoxin therapy suggests that the results are of value in assessing the degree of digitalization at the time of death. Control cases gave results within the normal therapeutic range whereas of six cases in which digoxin was suspected of being implicated in the death five had 'serum' digoxin levels above the therapeutic range. Differences in digoxin concentration were noted in blood collected from three sites in the body, and it is suggested that postmortem blood should be collected from the leg veins if assessment of antemortem digitalization is to be made.

The use of plasma and serum levels of digoxin in the therapeutic management of patients on maintenance doses of the drug is increasingly practised. Many workers in this field have found a statistical correlation between digoxin concentration and symptoms of digoxin toxicity (Smith *et al*, 1969; Beller *et al*, 1971). Experience in this laboratory agrees with that elsewhere in indicating that the majority of patients with plasma levels of digoxin in excess of 2 ng/ml show some symptoms of toxicity (Smith and Haber, 1970). The use of the rapid and sensitive radioimmunoassay for digoxin has enabled clinicians to evaluate the plasma or serum level of the drug in the light of other variables such as renal function and cation balance, and has led to a more precise administration of the drug in cases of equivocal toxicity (Oliver *et al*, 1971).

All pathologists are familiar with the problems of sudden death from cardiovascular causes. Necropsy may reveal coronary arteries in which one's principal surprise is that the patient should have survived for so long and in which the myocardium may show features ranging from no abnormality to fresh infarction or general or localized fibrosis of long standing. In many cases a characteristic history, together with the complete absence of suspicious circumstances, makes it reasonably certain that the patient has died from a cardiovascular cause, probably through the mechanism of fibrillation. When the patient has received digoxin before death there have been obvious clinical indications for its use, but it may be helpful to know the 'blood' digoxin level *post mortem*, especially where pathognomonic cardiovascular lesions are absent and there is no morbid anatomical cause of death.

The following preliminary findings are a postmortem assessment of cases taking into account the possibility of digoxin toxicity as a factor in the cause of death.

Received for publication 31 December 1974.

Methods

Serum digoxin levels were measured using radioimmunoassay with labelled tracers of either ^{3}H or ^{125}I-digoxin.

PROCEDURE WITH ^{3}H-DIGOXIN

To 250 μl of 'serum' sample, or standard, 500 μl of 0·067 M phosphate buffer pH 7·6 were added, followed by 50 μl (1 ng) of ^{3}H-digoxin specific activity 5Ci/mM (NEN, Boston, USA) and 50 μl of suitably diluted digoxin-specific antibody raised in this laboratory. Incubation was for 30 minutes at room temperature, after which 200 μl of BSA coated charcoal in barbitone buffer was added. Five minutes later the mixture was centrifuged at 3000 g for 15 minutes and an aliquot of the supernatant was transferred to a glass scintillation vial. Ten ml of a dioxan-based scintillator were added, after which the vials were heated in a waterbath for 10 minutes at 60°C and then centrifuged for 5 minutes at 3000 g. ^{3}H counting was carried out in a Packard model 3320 liquid scintillation spectrometer.

Correction for colour quench was effected by use of an internal standard of tritiated water.

The procedure using the ^{125}I tracer was largely the same, but since the radiolabelled digoxin was of a

9-45

Downloaded from jcp.bmj.com on 6 January 2009

higher specific activity — 500 Ci/mM (Wellcome Reagents Ltd, Beckenham, England) — sample volumes of 100 µl were used with 50 µl of labelled digoxin containing 200 pg. After charcoal addition and centrifugation an aliquot of the supernatant was transferred to a plastic tube for scintillation counting in an LKB/Wallac 80000 gamma sample counter.

Standards for the assay were prepared in fresh citrated human plasma in the range 0-4 ng/ml using a standard solution made from crystalline digoxin (Wellcome Reagents Ltd).

All standards were assayed in duplicate and samples in triplicate.

The iodinated tracer method was used preferentially as it was faster and required less counting time and no correction for colour quench. Samples assayed by both methods gave the same results to within 4%.

Results

Sixteen necropsies in which the patient had been receiving digoxin therapy were investigated. Ten were regarded at necropsy as 'controls' in which there was no reason to regard digoxin as having played any part (other than beneficial!). In six cases there was a suspicion that digoxin might have been a contributory factor in the cause of death. All 16 patients had been on maintenance digoxin therapy for a considerable period before death.

The six suspicious cases could be divided into two groups, and these together with the controls were so designated at necropsy and before the results of analysis were known.

The classification of the suspicious cases into two groups comprised:

GROUP 1

In these cases there was a high degree of probability that digoxin was implicated in the cause of death.

Case 1 BW, a 62-year-old woman. 0·25 mg digoxin daily. Slight mitral stenosis. Microscopy revealed small areas of fibrosis consistent with old rheumatic carditis. Death occurred in bed.

Case 2 AJ, a 74-year-old woman. 0·5 mg digoxin daily. Heart weight 15 oz. Slight thickening of the left ventricle though representative sections showed no abnormal features. Death occurred in bed.

Case 3 JA, a 63-year-old man. 0·25 mg digoxin daily. Moderate myocardial fibrosis. Heart chambers enlarged. Death sudden while ambulatory.

Case 4 RR, a 79-year-old man. 0·25 mg digoxin daily. Heart weight 13 oz. Both lungs affected by emphysema. Death occurred suddenly while seated in a chair.

GROUP 2

In these cases there was a possibility that digoxin had been a contributory factor in the cause of death but there was reasonable inference from the organs and history that death could have resulted solely from a cardiovascular cause.

Case 1 ES, a 66-year-old woman. 0·0625 mg digoxin daily. Heart weight 19 oz. Left ventricle thickened and much dilated. Death occurred in bed.

Case 2 AH, an 80-year-old woman. 0·25 mg digoxin daily. Heart weight 14 oz. Dense patchy fibrosis in the posterior wall of the left ventricle. Moderate atheroma of the coronary arteries. Death occurred suddenly while seated.

Blood was collected from the femoral vein into a glass vial and was centrifuged at 3000 g for 5 minutes. The supernatant, designated 'serum', was then assayed for digoxin. The results for the analysis of digoxin are shown in table I.

It can be seen that cases in group 1 had markedly elevated digoxin levels, while in group 2 ES was in the therapeutic range (although a little high in relation to the daily dose) and AH was within the toxic range.

	Patient	*Digoxin (ng/ml)*
Group 1		
	BW	4·0
	AJ	7·5
	JA	5·6
	RR	3·5
Group 2		
	ES	1·7
	AH	2·9
		Mean 4·2

Table 1 *Serum digoxin concentrations—'suspect' deaths*

CONTROL CASES

Cases were selected to check the possibility that post-mortem levels of digoxin were, as a rule, abnormally high. Samples were collected from digitalized cases where the death was certified without hesitation, usually from a cardiovascular cause, and where there was no suggestion that digoxin might be implicated. To monitor an additional variable it was decided to collect blood from three sites in the body to see whether differences in drug concentration existed depending on the site of sampling. Blood samples were collected from the femoral vein by milking into the vial, from the right ventricle of the heart by opening it in situ and allowing the blood to flow into the vial, and from the neck by allowing venous blood to flow into the vial as the skin was reflected.

Downloaded from jcp.bmj.com on 6 January 2009

Patient	*Heart*	*Neck*	*Leg*
Control cases			
EC	1·5	1·6	0·7
TD	1·9	1·7	0·8
RN	1·3	1·3	1·0
WL	1·8	2·0	1·2
MC	3·9	3·8	2·9
NS	3·7	2·5	2·1
JB	2·0	0·6	0·9
EH	2·0	1·6	1·2
18854	1·4	1·3	1·1
CB	3·3	2·0	1·9
Mean	2·33	1·84	1·38
'Suspect' cases			
RR	4·2	4·0	3·5
ES	3·7	3·6	1·7
AH	4·0	3·8	2·9

Table II *Serum digoxin concentrations (ng/ml)*

The results are shown in table II together with results for samples from all three sites obtained from three of our 'suspect' deaths.

Notably, in all cases but one, the level of digoxin in blood from the leg vein was markedly lower than the figures for the other two sites, and there was a tendency for that from the heart to be the highest of all. With the possible exception of patient MC, the digoxin level in blood from the leg was within the normal therapeutic range for the drug.

The results for samples collected from all three sites in 'suspect' deaths followed the same pattern as our 'control' cases.

There was a statistical difference between mean digoxin concentrations in blood from the leg and heart in our 'control' cases ($P < 0.005$ Student's t test) and between mean levels in our 'suspect' and 'control' cases for blood collected from the leg (figure).

The state of the samples obtained varied from those yielding a clear straw serum to those which were severely lysed, and it was thought prudent to evaluate the effect of haemolysis on the result given by radioimmunoassay.

Blood samples were drawn from two patients on maintenance digoxin therapy and from one volunteer taking no digoxin. An aliquot of the sample was heparinized and centrifuged while the remainder was subjected to ultrasonic shock treatment for 45 seconds using a Polytron PT200D homogenizer. The lysed blood was then centrifuged for 30 minutes at 25 000 g. The lysed blood, together with the plasma from the whole blood, was then assayed for digoxin using ^{125}I-labelled digoxin with the results shown in table III.

The distribution coefficient for digoxin between red cell/plasma in humans is 0·95 (Abshagen *et al*, 1971), and these results correlated well with the expected reduction in plasma level on complete haemolysis of the sample.

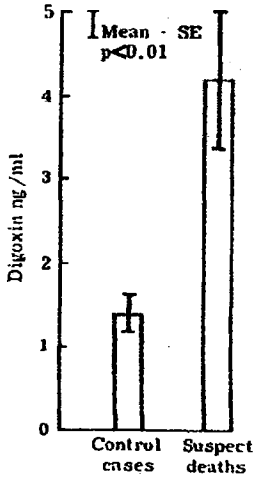


Figure *Mean digoxin concentrations in blood collected from the leg.*

Patient	*Plasma*	*Lysed Blood*
1	2·1	1·85
2	1·2	1·05
Control	Zero	Zero

Table III *Effect of haemolysis on digoxin concentration (ng/ml)*

The results for the blank sample confirmed that no substances were released which interfered with the assay when haemolysis occurred. As an additional check, samples were collected from the heart, neck, and leg in a case for which there was no record of antemortem digoxin ingestion; all three samples were negative for digoxin.

Samples assayed by both the ^{3}H and ^{125}I tracer methods gave results which showed no statistical difference between the methods and which were similar to previous published results comparing the two tracers (Drewes and Pileggi, 1974).

Discussion

High levels of digoxin (above the normal therapeutic range) were encountered in postmortem blood collected from the leg in five of our six cases

Downloaded from jcp.bmj.com on 6 January 2009

in which there was a suspicion that digoxin could have been implicated in the cause of death.

That these results represent genuinely high levels before death was borne out by our control cases in which levels within the normal therapeutic range for digoxin were encountered in blood taken from the leg.

The high levels obtained in cases from group 1 were well into the toxic range, and one case, A J, would qualify as an overdose. Such levels might constitute a lethal hazard since it is well documented that the toxic manifestations of digoxin include the precipitation of intractable congestive cardiac failure and the development of life-threatening arrhythmias (Chung, 1969; Fisch, 1971).

Our results also showed that there could be considerable variation in the digoxin concentration, depending upon the site from which blood is collected, with as much as 137% difference between blood from the heart and blood from the leg, the heart level being consistently higher.

It is possible that after death a new equilibrium between the blood and tissues is established, resulting in higher digoxin levels in blood collected from the heart, a tissue which per unit mass has a higher digoxin concentration than skeletal muscle (Doherty *et al*, 1967).

The finding that the drug level was almost always higher in blood collected from the neck compared with blood collected from the leg is not explained although antemortem differences in tissue distribution between those two areas seem to be the most likely explanation. Such postmortem differences have also been noticed for barbiturates (Gee *et al*, 1974) and paracetamol (Gee, 1974).

Our comparison of digoxin concentrations in plasma and haemolysed whole blood suggests that the degree of haemolysis of the samples does not significantly affect the result, nor does the process of lysing release compounds which interfere with the assay.

The implications of these findings are that the postmortem assay of digoxin can be used to investigate cases in which it is suspected that digoxin may have been a contributory factor in the cause of death. Levels above the normal therapeutic range appear to reflect elevated levels before death, but control samples suggest that blood from the leg should be used when retrospective use of the results is to be made.

On the basis of these results it appears that some patients who have been on digoxin therapy for some time may be 'at risk', having plasma levels of the drug well in excess of the normal therapeutic range. Such toxic plasma levels might well develop over a period of time as the result of a gradual reduction in renal function (probably the main determinant of plasma digoxin level) or because of erratic patient compliance in drug dosage.

Our study of 'suspect' deaths is continuing, together with an evaluation of digitalization among patients on long-term digoxin therapy in general practice.

We should like to thank Professor Gee, Department of Forensic Medicine, University of Leeds, for samples from case 18854 and Dr Ian Calder, St George's Hospital, London for the sample and case details of patient JA. Our grateful thanks are due to Dr P. O'Gorman, Department of Chemical Pathology, Greenwich District Hospital for the use of gamma counting facilities and to R. A. Lloyd, Esq, HM Coroner.

References

Abshagen, U., Kewitz, H., and Rietbrock, N. (1971). Distribution of digoxin, digitoxin and ouabain between plasma and erythrocytes in various species. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **270**, 105-115.

Beller, G. A., Smith, T. W., Abelmann, W. H., Haber, E., and Hood, W. B., Jr. (1971). Digitalis intoxication: a prospective clinical study with serum level correlations. *New Engl. J. Med.*, **284**, 989-997.

Chung, E. K. (1969). *Digitalis Intoxication*. Excerpta Medica Foundation, Amsterdam.

Doherty, J. E., Perkins, W. H., and Flanigan, W. J. (1967). The distribution and concentration of tritiated digoxin in human tissues. *Ann. intern. Med.*, **66**, 116-124.

Drewes, P. A. and Pileggi, V. J. (1974). Faster and easier radioimmunoassay of digoxin. *Clin. Chem.*, **20** (3), 343-347.

Fisch, C. (1971). Digitalis intoxication. *J. Amer. med. Ass.*, **216**, 1770-1773.

Gee, D. J. (1974). In *The Poisoned Patient: the Role of the Laboratory: Ciba Foundation Symposium* **26**, p. 224. Elsevier, Excerpta Medica, North Holland.

Gee, D. J., Dalley, R. A., Green, M. A., and Perkins, L. A. (1974). Post-mortem diagnosis of barbiturate poisoning. In *Forensic Toxicology*, edited by B. Ballantyne. Wright, Bristol.

Oliver, G. C., Parker, B. M., and Parker, C. W. (1971). Radioimmunoassay for digoxin. *Amer. J. Med.*, **51**, 186-192.

Smith, T. W., Butler, V. P., Jr., and Haber, E. (1969). Determination of therapeutic and toxic serum digoxin concentrations by radioimmunoassay. *New Engl. J. Med.*, **281**, 1212-1216.

Smith, T. W. and Haber, E. (1970). Digoxin intoxication: the relationship of clinical presentation to serum digoxin concentration. *J. clin. Invest.*, **49**, 2377-2386.

Interpretation of Excessive Serum Concentrations of Digoxin in Children

GIDEON KOREN, MD, and RUTH PARKER, MD

Between January 1981 and April 1984, excessive serum concentrations of digoxin (5 ng/ml or higher) were recorded in 47 children, aged 2 days to 16 years. In 10 patients, the high concentrations were measured 9.25 to 48 hours after death and were significantly higher than antemortem levels in all cases (8.3 ± 2.4 (± standard deviation) postmortem vs 3.3 ± 1.5 antemortem, <0.0001). In 15 patients (40.5% of the living patients) serum concentrations of 5 ng/ml or higher reflected sampling errors; drug levels were monitored too closely to the administration of a dose. None of these children had toxic manifestations of digoxin. In 10 patients, the excessive concentrations were associated with renal failure and a prolonged elimination half-life ($T^1/_2$) of digoxin; in 3 of these patients, there were signs of digoxin toxicity. Six cases were caused by digoxin overdose (accidental ingestions, pharmacy error and a suicide attempt). In 6 additional cases, the existence of an endogenous digoxin-like substance (EDLS) was shown to contribute to the excessive levels of the drug. One case could be attributed to digoxin-amiodarone interaction. In 10 of 37 living patients, digoxin toxicity was diagnosed. After excluding the 15 sampling errors and 6 cases with EDLS, this represents 63% of the cases. There was a good correlation between digoxin elimination $T^1/_2$ and serum creatinine concentrations ($r = 0.71$, $p < 0.01$). The above observations suggest that excessive serum concentrations of digoxin may not necessarily reflect potentially toxic levels. Sampling errors, postmortem determinations and circulating EDLS should be considered as explanations when toxic levels of digoxin are found.

(Am J Cardiol 1985;55:1210–1214)

Digoxin is one of the most ancient drugs in contemporary medicine. However, despite 2 centuries of clinical use, its use remains controversial.[1,2] Because of its narrow margin of safety, digoxin serum concentrations must be repeatedly monitored during chronic treatment. In adults, the therapeutic range is 1 to 2.5 ng/ml. However, children are believed to be less sensitive to digoxin and need higher doses.[3,4] However, excessive serum concentrations of the cardiac glycoside should not automatically be interpreted as reflecting toxicity. In the present studies, we reviewed cases of excessive serum digoxin concentrations in children to identify the causes of these levels.

From The Division of Clinical Pharmacology, The Research Institute, Department of Pediatrics, The Hospital for Sick Children, Toronto, Ontario, Canada, and Strong Memorial Hospital, Rochester, New York. Manuscript received November 8, 1984; revised manuscript received and accepted January 14, 1985.

Address for reprints: Gideon Koren, MD, Division of Clinical Pharmacology, Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada.

Methods

To identify excessive serum determinations of digoxin, the Therapeutic Drug Monitoring Laboratory charts of digoxin at the Hospital for Sick Children in Toronto were screened for the period between January 1981 and April 30, 1984. All assays were performed by a radioimmunoassay (New England Nuclear until the end of March 1983 or TDX (Abbot, Ltd.) since April 1983). The coefficient of variation for the tests in this laboratory is less than 5% for levels above 1 ng/ml. An "excessive level" was arbitrarily defined as 5 ng/ml or higher, because in children, some investigators believe that toxicity occurs at levels higher than the adult range of 1 to 2.5 ng/ml.[4] A level of 5 ng/ml or greater, on the other hand, is not controversial in this respect. The charts of the children in whom serum concentrations were 5 ng/ml or higher were reviewed. Details of their ages, weights, diseases, renal function, digoxin and other drug therapy were obtained.

Digoxin toxicity was defined by clinical signs (anorexia, nausea, vomiting, bradycardia, arrhythmia and abdominal pain) and electrocardiographic signs. Digoxin elimination half-life was determined by least-squares linear regressions of the concentration-time data after stopping the drug, plotted on semilogarithmic paper. Serum digoxin concentrations determined in patients after they died compared with ante-

9-49

PLAINTIFFS' EXHIBITS 012631

mortem levels by the Student *t* test for paired results in 10 children. The correlation between digoxin elimination T½ and creatinine serum concentration was assessed by least-squares linear regression.

Results

Between January 1981 and April 1984, serum digoxin concentrations of 5 ng/ml or higher were detected in 47 children. They were 2 days to 16 years old. Table I is a list of the various factors that contributed to the excessive levels. In some children, more than 1 factor could be implicated, and in 4 children the high levels of digoxin could not be explained by any of the putative mechanisms.

Postmortem serum concentrations of digoxin: In 10 infants, all of whom had inoperable congenital heart disease, postmortem levels were significantly higher (8.3 ± 2.4 ng/ml) than antemortem levels (3.3 ± 1.5 ng/ml) ($p < 0.0001$) (Fig. 1). The postmortem determinations were performed 9.25 to 48 hours after death. No correlation was found between the length of time elapsed until the postmortem determination and the rate of elevation in serum digoxin concentration.

Sampling error: In 15 instances, digoxin levels of 5 ng/ml or higher could be well explained by sampling blood 5 minutes to 3 hours after a dose (peak level). Subsequently, the dose was discontinued and repeated assessment failed to reveal excessive levels. None of these children had clinical signs of digoxin toxicity.

Case 1: A 4-month-old girl suffering from atrioventricular canal, cleft mitral valve and congestive heart failure was treated intravenously with digoxin in a dose of 4 μg/kg twice daily. She appeared to benefit from the drug and previous serum concentrations recorded before a dose were 1.5 to 2 ng/ml. One morning a level of 6.2 ng/ml was recorded, and digoxin therapy was stopped despite the potential benefit and lack of toxic signs. Twenty-four hours later, the digoxin level was 1.7 ng/ml. Investigation revealed that the excessive concentration was erroneously measured 20 minutes after the administration of her morning dose.

Overdose: Overdose could clearly be determined in 6 cases, 4 of which occurred outside the hospital. Two infants swallowed an undetermined number of digoxin tablets that belonged to family members. In 1 case, a child was given an excessive dose of Lanoxin® syrup because of a pharmacy labeling error. A 15-year-old girl consumed 32 tablets of her father's digoxin during a suicide attempt. Because of induced emesis and charcoal ingestion, it was impossible to determine how much of the drug was eventually absorbed.

Renal insufficiency: In 10 instances, renal insufficiency was evident at the time of the excessive serum concentration of digoxin. Three patients had end-stage kidney diseases; however, the digoxin dose was not reduced and dosing intervals were not prolonged to adjust for the renal disease. Acute digoxin toxicity occurred in 3 patients. In a few other cases, acute renal insufficiency was not considered in adjusting digoxin dosage.

Endogenous digoxin-like substance (EDLS): Six newborn babies and infants showed evidence of circulating EDLS, which could partially explain readings of excessive digoxin. All six were critically ill, and in 4 patients, there was associated acute renal failure (Table II). Because of high serum concentrations, digoxin therapy was stopped; however digoxin levels as measured by the routine radioimmunoassay continued to rise in 4 of them. In 2 other patients, after an initial decrease in serum concentrations, the digoxin level eventually "plateaued" despite cessation of therapy for a few days.

Case 2: A critically ill 5-week-old boy with severe aortic stenosis, cardiac failure and endocarditis was treated with digoxin, 10 μg/kg twice daily, and his measured serum concentration was 1.8 ng/ml. Two days later, during deterioration of his general condition, a predose level was 6 ng/ml, and consequently digoxin therapy was stopped. However, 2 days later, a few hours

TABLE I Mechanisms Involved in Excessive (≥5 ng/ml) Serum Concentration of Digoxin in 47 Children

	No. of Children*
High postmortem levels	10
Sampling error	15
Overdose	6
Renal insufficiency	10
Endogenous digoxin like substance	6
Digoxin-amiodarone interaction	1

* In some children, more than 1 mechanism could be shown (e.g, renal failure and endogenous digoxin-like substances), whereas in 4 cases, the high measured level of digoxin could not be explained by any of the above factors.

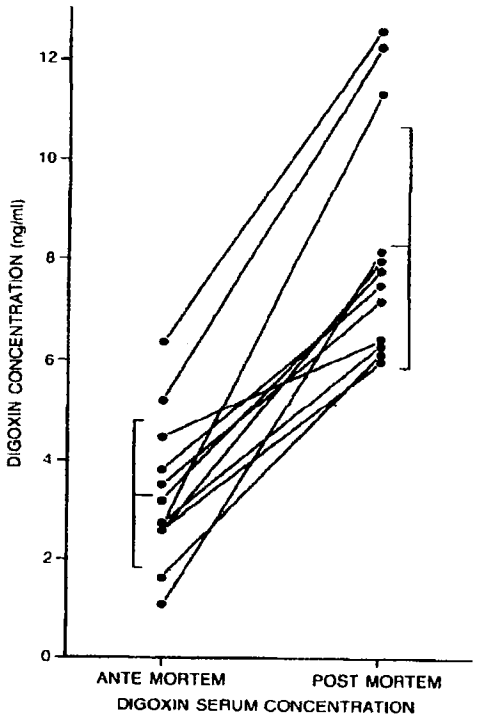


FIGURE 1. Postmortem concentrations of digoxin are significantly higher than antemortem concentrations ($p < 0.0001$).

9-50

TABLE II Characteristic of Six Infants Who Had Evidence That Endogenous Digoxin-Like Substances Contributed to Excessive Digoxin Readings

Pt	Wt (kg)	Age (days)	Diagnosis	Highest Serum Digoxin Reading (ng/ml)
A.L.	0.51	21	PDA, RDS	6.0
C.M.	3.2	37	RDS, aortic stenosis	11.4
C.L.	4.0	7	Septic shock, RI	6.6
C.Z.	5.2	60	Endocardial fibroelastosis, RI	5.2
E.C.	3.8	20	Multiple thrombi, RI	7.2
S.C.	5.5	270	AV canal, RI, Down's syndrome	6.2

AV = atrioventricular; PDA = patent ductus arteriosus; RDS = respiratory distress syndrome; RI = renal insufficiency; SBE = subacute bacterial endocarditis.

before his death, the serum digoxin concentration was 11.4 ng/ml.

Case 3: A 9-month-old boy with Down's syndrome and atrioventricular canal was referred from another hospital because of deep coma secondary to an erroneous overdose of morphine. On admission, a serum digoxin concentration of 6.2 ng/ml was found, and digoxin therapy was discontinued. During the following days, the serum digoxin concentration slowly decreased (with a $T^1/_2$ of 64 hours), corresponding to transient renal failure. However, after reaching a level of 1.3 ng/ml, serum digoxin concentration stayed unchanged at levels between 1.3 and 1.5 ng/ml for an additional week, although digoxin was not administered.

Interaction of digoxin with other drugs: A case of amiodarone-associated digoxin toxicity has been reported elsewhere.[5] Digoxin-quinidine interaction has been reported in a group of children and may cause toxic signs[6]; however, none of the patients in these studies had a level of 5 ng/ml or higher. Similarly, in a group of newborn infants with patent ductus arteriosus, serum digoxin concentrations were acutely elevated after administration of indomethacin, although digoxin levels did not reach 5 ng/ml.[7]

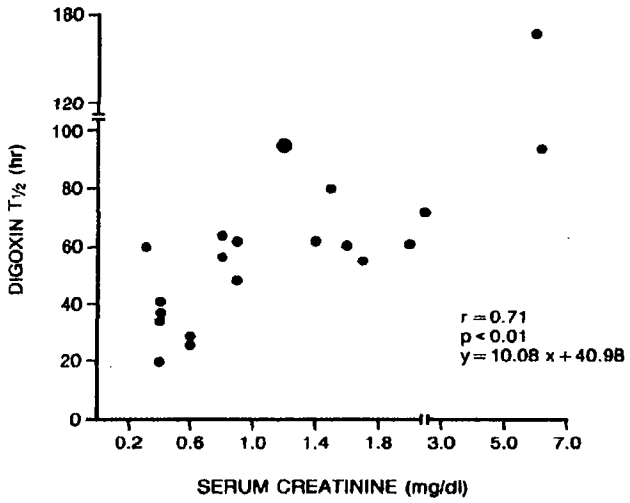


FIGURE 2. Good correlation between serum creatinine concentration and digoxin elimination half-life ($T^1/_2$). The circled point represents a child with digoxin-amiodarone interaction; despite relatively adequate renal function there was a prolonged $T^1/_2$.

Digoxin toxicity: Ten patients had evidence of digoxin toxicity (Table III). Nine of these patients had digoxin overdose or renal insufficiency. In some of the more critically ill children, digoxin toxicity may have been masked by their generalized disease. In none of the cases of high digoxin concentrations resulting from erroneous sampling were there signs of digoxin toxicity. After excluding these 15 cases and an additional 6 cases of EDLS, digoxin toxicity could be diagnosed clinically in 63% of cases with serum digoxin concentrations of 5 ng/ml or higher.

Digoxin elimination half-life: In 19 cases, there were sufficient data to calculate the correlation between serum creatinine concentration and digoxin elimination $T^1/_2$ ($r = 0.71$, $p < 0.01$) (Fig. 2). One child had a digoxin-amiodarone interaction; despite relatively adequate renal function there was a prolonged $T^1/_2$ of digoxin, presumably because of inhibition of the renal tubular secretion of digoxin without affecting glomerular filtration rate.[5]

Discussion

The arbitrary cutoff value of 5 ng/ml that we chose does not imply that digoxin toxicity cannot occur at lower levels.[8] Halkin et al[9] found electrocardiographic toxic signs in 4 out of 11 neonates and infants who had digoxin levels higher than 2 ng/ml. Lanese and Mizkin,[10] on the other hand, found no relation between serum concentrations and onset or persistence of cardiac arrhythmias.[10] However, in our attempt to interpret excessive digoxin levels, we had to choose a level that is identified by all clinicians as potentially toxic. Several mechanisms could be positively identified as causing or contributing to excessive serum concentrations of the cardiac glycoside. Postmortem levels were significantly higher than antemortem levels in all children studied (Fig. 1). These results are consistent with previous reports,[11,12] suggesting that after death, redistribution of digoxin takes place. We recently reproduced these results in rats, showing that after death, digoxin reenters the blood from various tissue compartments, presumably because of cessation of the active accumulation of the glycoside that occurs during life.[13] These observations may have important medicolegal implications. An attempt to prove digoxin intoxication as a cause of death

may be hampered by the fact that postmortem levels may be 1.5 to 10 times higher than antemortem levels. Consequently, one cannot readily use these postmortem data to predict antemortem concentrations. Only if postmortem concentrations are in the therapeutic range can one assume that antemortem concentrations were not excessive.

The high incidence of sampling errors (43% of living cases) was surprising. In all these cases a digoxin level of 5 ng/ml or higher was interpreted as potentially toxic, and the drug was discontinued for varying lengths of time. After intravenous, intramuscular or oral administration of digoxin, large amounts of the drug circulate in the blood. The distribution of digoxin is relatively slow, and eventually only about 1% of the dose stays in the blood, the rest being distributed into muscle, liver, kidney and skin.[12] Consequently, a post-dose sampling may yield extremely high levels, which do not correlate with or reflect toxicity. In none of these cases were there signs of digoxin toxicity.

Digoxin overdose appeared to be the single most common cause of digoxin toxicity, accounting for 60% of the cases of verified toxicity in the present study (Table III). Children with normal hearts who were exposed to the drug could tolerate serum concentrations of 15 ng/ml relatively well, and did not have signs of toxicity when levels decreased to 6 ng/ml. On the other hand, children with compromised hearts showed signs of toxicity when levels reached 5 to 6 ng/ml. Other factors, including hypoxia, hypokalemia, hypercalcemia, hypomagnesemia, acid-base disturbances and administration of sympathomimetic amines, may precipitate *digoxin intoxication*.[8] Toxic symptoms such as visual disturbances and malaise reported in adults are difficult to judge in young children.[8] Nausea and persistent vomiting, on the other hand, are frequent manifestations in children. These characteristics are reflected in our patients (Table III). Similar to previous reports in children, most of our patients with signs of toxicity had atrial arrhythmias.[8]

In humans, digoxin is eliminated almost entirely unchanged by the kidney through both glomerular filtration and tubular secretion.[13] This association is clearly documented by the correlation between the elimination $T\frac{1}{2}$ of digoxin and creatinine serum concentration. Several exceptions must be taken into account: Several drugs, including the antiarrhythmic agents quinidine, verapamil and amiodarone decrease renal clearance of digoxin without affecting glomerular filtration rate[6]; consequently, they may cause accumulation and toxicity of digoxin. In such cases, prolongation of digoxin $T\frac{1}{2}$ will not be accompanied by an increase in serum creatinine concentration. This is exemplified in the present study by the child who had digoxin toxicity associated with amiodarone (Fig. 2, circled point). Whenever one of these drugs is coadministered with digoxin, a careful assessment of digoxin serum concentration should be carried out with appropriate reduction of the dose to avoid toxicity.

During renal failure, both clearance and volume of distribution of digoxin are decreased, and therefore the loading and maintenance dose should be significantly reduced and the dosing interval prolonged.

TABLE III Clinical and Electrocardiographic Manifestations of Digoxin Toxicity in 10 Children

	No. of Pts
Mechanisms of accumulation	
Overdose	6
Renal insufficiency	3
Digoxin-amiodarone interaction	1
Clinical signs	
Anorexia	4
Nausea	4
Vomiting	4
Lethargy	3
Congestive heart failure	2
Electrocardiographic signs	
Sinus bradycardia	3
Atrial escape beats	1
Cardiac arrest	2
Changes in ST segment	2
Wenckebach phenomenon	1
1st-degree atrioventricular block	3
Nodal rhythm	1
Bigeminy	1
Idioventricular rhythm	1

The possible existence of EDLS in 6 of our patients is of particular interest. Previous studies show that EDLS exists in the blood of a majority of preterm infants[14–16]; however, none of the infants in these studies was receiving digoxin. Unexplained elevation of digoxin levels in critically ill adults with renal failure has been attributed to EDLS.[17] Our children with EDLS continued to have increasing digoxin levels long after discontinuation of cardiac glycoside therapy without evidence of digoxin toxicity. In other cases, digoxin serum concentrations decreased to a "plateau" level that was maintained for long periods. These phenomena might have been partially explained by acute changes in distribution volume and clearance; however, even in renal failure digoxin levels decrease gradually and do not increase.[18] Moreover, these possible changes cannot explain the residual steady readings of digoxin long after stopping treatment. Until an assay is available that can differentiate between digoxin and EDLS, it is advisable to measure a pretreatment level of digoxin in newborn infants or critically ill children. This may yield some information on EDLS levels; however, these levels are not stable over time, and a simple subtraction of pretreatment EDLS level from apparent reading of digoxin during treatment may not yield the "true" level of digoxin.

Acknowlegment: We thank Linda Citren and Margaret Armstrong for excellent secretarial assistance.

References

1. White RD, Liebman PS. A re-appraisal of digitalis for infants with "left-to-right shunts" and "heart failure." J Pediatr 1978;92:867–870.
2. Marrow CD, Fessenner JR, Velez R. Reevaluation of digitalis efficacy: light on an old leaf. Ann Intern Med 1984;101:113–116.
3. Kearin M, Kelly JG, O'Malley K. Digoxin "receptors" in neonates: an explanation of less sensitivity to digoxin than in adults. Clin Pharmacol Ther 1980;28:346–349.
4. Morselli PL, Morselli RF, Bossi L. Clinical pharmacokinetics in newborn and infants. In: Gibaldi M, Prescott L, eds. Handbook of Clinical Pharma-

9-52

Case 2:08-md-01968 Document 580-27 Filed 09/08/11 Page 42 of 64 PageID #: 24517

cokinetics. New York: ADIS Health Science Press, 1983:109–112.
5. **Koren G, Hesslein PS, MacLeod SM.** Digoxin toxicity associated with amiodarone therapy in children. J Pediatr 1984;467–470.
6. **Koren G.** Interaction between digoxin and commonly coadministered drugs in children, Pediatrics, in press.
7. **Koren G, Zarfin Y, Perlman M, MacLeod SM.** Effects of indomethacin on digoxin pharmacokinetics in preterm infants. Pediatr Pharmacol 1984;4: 25–30.
8. **Gorodischer R.** Cardiac drugs. In: Yaffe SJ, ed. Pediatric Pharmacology, New York: Grune & Stratton, 1980:269–290.
9. **Halkin H, Radomsky M, Blieden L, Frand M, Bolchis H.** Steady state serum concentration in relation to digitalis toxicity in neonates and infants. Pediatrics 1978;61:184–188.
10. **Larese RJ, Mirkin BL.** Kinetics of digoxin absorption and relation of serum levels to cardiac arrhythmias in children. Clin Pharmacol Ther 1974;15: 387–396.
11. **Vorphal TE, Coe JI.** Correlation of antemortem and postmortem digoxin levels. J Forens Sci 1978;23:329–334.
12. **Karjalainen J, Ojala K, Reissell P.** Tissue concentrations of digoxin in an autopsy material. Act Pharmacol Toxicol 1974;34:389–390.
13. **Koren G, MacLeod SM.** Postmortem redistribution of digoxin in rats. J Forens Sci 1985;30:92–96.
14. **Pudek MR, Seccombe DW, Whitefield MF.** Digoxin-like immunoreactivity in premature and full-term infants not receiving digoxin therapy. N Engl J Med 1983;308:904–905.
15. **Koren G, Farine D, Maresky D, Taylor J, Heyes J, Soldin S, MacLeod SM.** Significance of the endogenous digoxin-like substance in infants and mothers. Clin Pharmacol Ther 1984;36:759–764.
16. **Valdes R, Graves SW, Brown BA, Landt M.** Endogenous substance in newborn infants causing false positive digoxin measurements. J Pediatr 1983:102:947–950.
17. **Graves SW, Brown B, Valdes R.** An endogenous digoxin-like substance in patients with renal impairment. Am Intern Med 1983;99:604–608.
18. **Dettli L.** Drug dosage in renal disease. In: Gibaldi M, Prescott L, eds. Handbook of Clinical Pharmacokinetics. New York: ADIS Health Science Press, 1983:261–276.

PLAINTIFFS' EXHIBITS 012635

9-53

Articles

Incidence of Digoxin Toxicity in Outpatients

JOHN F. STEINER, MD, MPH; LAURENCE J. ROBBINS, MD; KARL E. HAMMERMEISTER, MD; STEPHEN C. ROTH, RN, MPA; *and* WILLIAM S. HAMMOND, MD, *Denver, Colorado*

The incidence of digoxin toxicity among patients in hospitals has declined in recent years. To evaluate whether a similar decline has occurred in ambulatory care, we reviewed randomly selected medical records for 183 outpatients receiving ongoing treatment with digoxin at 10 urban and rural Department of Veterans Affairs Medical Centers in the Rocky Mountain region. The prevalence of traditional risk factors for digoxin toxicity—elevated serum digoxin and serum creatinine levels, hypokalemia, and a new prescription of an interacting drug—was established from computerized laboratory and pharmacy records. Of the 183 patients, 50 (27.3%) had one or more risk factors for digoxin toxicity: serum digoxin levels were elevated in 13.6% of patients in whom a level was obtained, with hypokalemia in 14.3%, elevated creatinine levels in 17.9%, and possible drug interactions in 5.5% of patients over a 1-year period. Nevertheless, digoxin toxicity occurred in only 2 persons (1.1% or 1.4 per 100 patient-years of treatment). We conclude that digoxin toxicity was rare in this group of outpatients, even in persons presumed to be at high risk because of metabolic abnormalities, increased digoxin concentrations, or the use of interacting drugs. The low rate of digoxin toxicity in outpatients parallels the decline in the incidence of toxicity observed in hospital-based studies.

(Steiner JF, Robbins LJ, Hammermeister KE, Roth SC, Hammond WS: Incidence of digoxin toxicity in outpatients. West J Med 1994; 161:474-478)

As long as digoxin has been used in the treatment of congestive heart failure (CHF) and atrial arrhythmias, clinicians have been taught to anticipate a high incidence of digoxin toxicity. Studies published 10 to 20 years ago reported digoxin toxicity in 11% to 30% of patients receiving the drug at the time of hospital admission,[1-5] and the incidence of digoxin toxicity among less acutely ill outpatients has been estimated as 5 cases per 100 patient-years of treatment.[6,7] In more recent reports, however, the incidence of digoxin toxicity had fallen to 5% among patients admitted to hospital with CHF[8] and to 1.1% among closely monitored outpatients in a randomized trial.[9] Most studies that found a high rate of digoxin toxicity were conducted before the widespread use of serum digoxin concentrations to monitor drug treatment and before the development of alternative medications for CHF and atrial arrhythmias. These studies identified risk factors for digoxin toxicity in patients admitted to hospitals, such as elevated serum digoxin levels, hypokalemia, renal impairment, or the prescription of interacting medications.[10] They did not define the predictive value of these risk factors in outpatients, however—that is, the likelihood that digoxin toxicity would actually develop in outpatients with a given risk factor. To determine whether the incidence of digoxin toxicity has declined in ambulatory care practice and to establish the prevalence and predictive value of traditional risk factors for digoxin toxicity in outpatients, we conducted a retrospective cohort study among clinic patients receiving digoxin at ten Department of Veterans Affairs (VA) medical centers in the Rocky Mountain region.

Methods

In December 1988, the VA quality assurance organization, the Medical District-Initiated Peer Review Organization (MEDIPRO), authorized a study of digoxin use in the ten medical centers in VA Medical District 23. These facilities (in Cheyenne, Wyoming; Denver, Colorado; Fort Harrison, Montana; Fort Lyon, Colorado; Fort Meade, South Dakota; Grand Junction, Colorado; Hot Springs, South Dakota; Miles City, Montana; Salt Lake City, Utah; and Sheridan, Wyoming) all provide inpatient acute care and outpatient primary care. Two sites (Denver and Salt Lake City) also provide tertiary referral care and

From the Department of Medicine, Divisions of General Internal Medicine (Dr Steiner), Geriatrics (Dr Robbins), and Cardiology (Dr Hammermeister), Denver Department of Veterans Affairs Medical Center; the Center for Health Services Research (Dr Steiner); the Department of Pathology, University of Colorado Health Sciences Center (Dr Hammond); and the Medical District-Initiated Peer Review Organization (MEDIPRO), Veterans Affairs Medical District 23 (Mr Roth), Denver, Colorado.

Support for this project was provided by the Veterans Health Services and Research Administration, Department of Veterans Affairs.

Reprint requests to John F. Steiner, MD, MPH, Center for Health Services Research, University of Colorado Health Sciences Center, 1355 S Colorado Blvd, Ste 706, Denver, CO 80222.

ABBREVIATIONS USED IN TEXT

AV = atrioventricular
CHF = congestive heart failure
CI = confidence interval
MEDIPRO = Medical District-Initiated Peer Review Organization
VA = [Department of] Veterans Affairs

are located in major metropolitan areas; the remainder are located in either rural communities or cities of less than 50,000 population.

Data Collection

From a pharmacy-generated list of all outpatients who received one or more digoxin prescriptions in the previous year, 20 patients from each facility were randomly selected for on-site review of medical records, computerized pharmacy profiles, and laboratory data. A trained MEDIPRO quality assurance nurse (S.C.R.) completed the chart reviews between August 1989 and February 1990. The reviewer recorded patient demographics and enumerated all outpatient visits and hospital admissions over the 12 months preceding the date of chart review. Atrial arrhythmias were confirmed as an indication for digoxin therapy only if documented by electrocardiograms obtained during the study year. The presence of CHF was assessed by a validated, 12-point CHF scale based on medical record evidence of symptoms (such as paroxysmal nocturnal dyspnea), abnormalities on a physical examination (for instance, the presence of a third heart sound), and findings from chest radiographs (such as alveolar pulmonary edema) during the study year.[6] Patients with a combination of findings totaling 5 or more points on this 12-point scale were defined as having CHF, a cutoff with a sensitivity of 90% and a specificity of 85% for detecting left ventricular failure as documented by an elevated resting pulmonary capillary wedge pressure.[6] The entire medical record for five patients at each site was reviewed to determine how often patients with no evidence of CHF during the study year had CHF in earlier years, explaining the continued use of digoxin.

Computerized laboratory records from the Decentralized Hospital Computing Program at the ten VA medical centers provided all serum digoxin concentrations, electrolytes, and serum creatinine determinations during the study year. To evaluate the prevalence of commonly accepted risk factors for digoxin toxicity, we identified all patients with serum digoxin levels of greater than 2.6 nmol per liter (2.0 ng per ml), serum potassium levels of less than 3.5 mmol per liter (3.5 mEq per liter), or serum creatinine levels of 176.8 μmol per liter (2.0 mg per dl) or greater. From pharmacy profiles, we identified new prescriptions for quinidine sulfate or gluconate, amiodarone, and verapamil hydrochloride, three medications that predictably and substantially increase serum digoxin levels.[10]

Methods for Identifying Digoxin Toxicity

The chart reviewer recorded all diagnoses of digoxin toxicity noted in the medical record. To identify additional cases of digoxin toxicity that lacked an explicit diagnosis in the chart, we conducted a supplemental chart review for all patients who had an elevated serum digoxin level or who reduced the dosage or stopped taking digoxin during

TABLE 1.—*Clinical Characteristics of Patients With Probable or Possible Digoxin Toxicity*

Patient	Age, yr	Reason for Digoxin Use	Digoxin Dose, mg	Possible Drug Interactions	Digoxin Level, nmol/liter (ng/ml)	Serum Potassium, mmol/liter	Serum Creatinine, μmol/liter (mg/dl)	Symptoms Suggestive of Digoxin Toxicity	ECG Evidence of Digoxin Toxicity	Response to Reduced Digoxin Dosage
Probable toxicity*										
1	67	CHF, atrial fibrillation	0.25	None	4.9 (3.8)	3.8	433 (4.9)	Decreased mental status	Slow atrial fibrillation (rate 30 to 60/min)	Mental status returned to normal; increase in heart rate (80 to 98/min)
2	69	CHF, atrial fibrillation	0.625	None	4.4 (3.4)	4.7	239 (2.7)	Vomiting	Normal	Vomiting resolved
Possible toxicity†										
3	68	CHF, atrial fibrillation	0.25	Newly prescribed verapamil	4.1 (3.2)	4.4	115 (1.3)	None	Slow atrial fibrillation (rate 49 to 51/min)	Increase in heart rate (70 to 86/min)
4	76	None apparent	0.25	None	2.7 (2.1)	4.7	80 (0.9)	Anorexia, weight loss	None	No follow-up data
5	46	CHF	0.25	Long-term amiodarone therapy	2.8 (2.2)	5.1	None	Fatigue	None	No follow-up data
6	69	Atrial fibrillation	0.125	None	None	4.5	80 (0.9)	None	Slow atrial fibrillation (rate 40 to 60/min)	Increase in heart rate (65 to 73/min)

CHF = congestive heart failure, ECG = electrocardiogram

*Patients 1 and 2 were identified as having "probable" digoxin toxicity in the medical record, confirmed by the aggregate opinion of the chart reviewers.
†Patients 3 to 6 were rated as having "possible" digoxin toxicity by two chart reviewers, but as having "probable" toxicity by none.

9-55

the study year. Medical records and electrocardiograms for these patients were independently reviewed by a general internist (J.F.S.), a geriatrician (L.J.R.), and a cardiologist (K.E.H.). The reviewers recorded any gastrointestinal, neurologic, or visual symptoms or electrocardiographic findings suggestive of digoxin toxicity—sinus bradycardia, sinus arrest, Mobitz I second-degree atrioventricular (AV) block, complete AV dissociation, AV junctional tachycardia, atrial tachycardia with block, unifocal or multifocal ventricular premature beats, ventricular tachycardia, ventricular fibrillation, atrial fibrillation or flutter with a ventricular response of less than 60 beats per minute, or atrial fibrillation with ventricular premature beats.[12] The reviewers also recorded whether symptoms or electrocardiographic abnormalities improved after the reduction or cessation of digoxin therapy. Each reviewer then estimated digoxin toxicity as "probable," "possible," or "unlikely." To reach an aggregate rating, we defined "probable" digoxin toxicity in any case rated as probably toxic by at least one reviewer and as possibly toxic by at least one other reviewer.

Statistical Methods

We calculated the person-years of treatment with digoxin from pharmacy records. The incidence of toxicity was calculated both as the number of toxic events divided by the number of study patients and the number of events divided by the person-years of treatment; 95% confidence intervals (CI) for the latter were determined from a table of exact confidence intervals for binomial proportions.[11] The predictive value of each traditional risk factor was calculated as the proportion of patients with the risk factor at any time during the study year in whom digoxin toxicity developed during that year.[12] Interrater reliability was measured by the kappa (κ) statistic, which corrects the proportion of observed agreement for the amount of agreement expected by chance alone. κ-Values from 0.0 to 0.20 represent "slight" agreement, 0.21 to 0.40 represent "fair" agreement, while 0.41 to 0.60 is "moderate," 0.61 to 0.80 is "substantial," and 0.81 to 1.0 is "almost perfect" agreement.[13]

Results

Of the 200 patients randomly identified for the study, medical records were available for 183 (91.5%). The mean age of the 183 patients was 69.1 ± 9.5 years; 98.9% were male. These patients had 7.1 ± 6.4 outpatient visits to the VA facility during the study year and 0.8 ± 1.3 hospital admissions for all causes. The mean digoxin dose prescribed was 0.20 ± 0.81 mg per day; 17 patients (9.3%) were prescribed doses greater than 0.25 mg per day. In all, 59 patients (32.2%) had electrocardiographic evidence of atrial arrhythmias—atrial fibrillation or supraventricular tachycardia—and 66 (36.1%) had CHF; 36 patients (19.7%) were admitted to hospital for atrial arrhythmias or CHF. Overall, 96 patients (52.5%) had one or both indications for digoxin use during the study year. We completed 48 full chart reviews to assess whether the one-year review underestimated the prevalence of CHF. This condition was present in 17 of these patients (35.4%), of whom 3 had evidence of CHF in previous years, but not during the study period. Thus, the use of the one-year chart review did lead to a modest underestimation of the actual prevalence of CHF in our sample.

The 183 study patients accumulated a total of 141.6 person-years of digoxin treatment during the study year. Only two cases of digoxin toxicity (1.1%; 1.4 per 100 patient-years on the drug; 95% CI, 0.4 to 5.5) were explicitly noted in the medical record. Neither of these patients died of digoxin toxicity. Both patients had increased serum digoxin concentrations and elevated serum creatinine levels at the time of their toxicity; neither had hypokalemia or a possible drug interaction with digoxin. These patients' clinical characteristics are described in Table 1 (patients 1 and 2). Medical records for the supplemental chart review to detect digoxin toxicity were obtained for 37 of the 38 patients (97%) who had serum digoxin concentrations of greater than 2.6 nmol per liter (12 patients) or who had reduced the dosage or stopped taking digoxin (32 patients). The two patients diagnosed with toxic reactions by their clinicians were included in this supplemental review. Reviewer A rated 6 of the 37 patients as possibly toxic and 1 as probably toxic, whereas reviewer B rated 7 patients as

TABLE 2.—*Prevalence of Risk Factors for Digoxin Toxicity*

Risk Factor	Patients With Sufficient Data for Evaluation* No.	%	Patients Ever Having Risk Factor† No.	%	Patients With ≥30 Days' Duration of Risk Factor† No.	%	Patients With Risk Factor(s) Having Probable Digoxin Toxicity No.	%
Possible drug interaction‡	182	99.5	9	5.5	7	3.8	0/9	0.0
Serum digoxin level >2.6 nmol/liter (2.0 ng/ml)	88	48.1	12	13.6	3	3.4	2/12	16.7
Serum creatinine level ≥176.8 μmol/liter (2.0 mg/dl)	145	79.2	26	17.9	14	9.7	2/26	7.7
Serum potassium level < 3.5 mmol/liter	147	80.3	21	14.3	4	2.7	0/21	0.0

*The number of patients in the study = 183.
†Proportions were determined from the total number of patients with measurement of that risk factor.
‡A possible drug interaction was defined as a new prescription of quinidine sulfate or gluconate, verapamil hydrochloride, or amiodarone for a patient already receiving digoxin.

possibly toxic and 2 as probably toxic, and reviewer C rated 2 patients as possibly toxic and 0 as probably toxic. The agreement among reviewers for the combination of possible-probable toxicity was 84% between reviewers A and B, 86% between reviewers A and C, and 76% for reviewer B with reviewer C. Agreement for probable toxicity alone was greater than 95% among all reviewers, due to the rarity of cases. Similar to previous research,[14] κ-statistics for possible or probable toxicity were 0.52 between reviewers A and B, 0.39 between reviewers A and C, and only 0.10 between reviewers B and C. The aggregate rating confirmed the clinical assessment of probable digoxin toxicity in patients 1 and 2 in Table 1. Four other patients were rated as possibly toxic by two reviewers, but as probably toxic by none. These cases are described as patients 3 to 6 in Table 1. Thus, the supplemental review ultimately identified no additional cases of digoxin toxicity.

Because digoxin toxicity was present in only 2 of the 32 patients who reduced the dosage or stopped taking the drug during the study year, we attempted to identify other reasons for the change in digoxin therapy. No reason for decreasing or stopping digoxin therapy was apparent in the medical records of 12 patients. The reasons for the step-down of digoxin in the remaining 18 patients included increased serum digoxin concentrations without evidence of toxicity (7 patients), the substitution of another drug for digoxin (3 patients), symptoms or electrocardiographic changes attributed to digoxin therapy that were not indicative of drug toxicity (3 patients), and a variety of reasons in the remaining 5 patients.

About 80% of patients had serum electrolyte or creatinine measurements during the study year; less than half (48.1%) were monitored with a serum digoxin level. The mean serum digoxin concentration for these patients was 1.3 ± 0.6 nmol per liter (1.0 ± 0.50 ng per ml). Of the study sample, 50 patients (27.3%) had at least one risk factor for digoxin toxicity during the study year, most commonly an elevated serum creatinine level (17.9%) or hypokalemia (14.3%) (Table 2). In most cases, risk factors appeared to be of short duration (Table 2), as only 9.7% of the patients had elevated serum creatinine levels, and 2.7% had hypokalemia on two consecutive determinations 30 days or more apart. Nine patients received new prescriptions for quinidine or verapamil, of whom only two either reduced their digoxin dosage or were monitored with a serum digoxin level within 14 days of starting the interacting drug. The proportion of patients with risk factor(s) in whom digoxin toxicity developed ranged from 0% for patients with hypokalemia (95% CI, 0.0% to 16.1%) or possible drug interactions (95% CI, 0.0% to 33.6%) to 16.7% (95% CI, 2.1% to 48.4%) among those with elevated serum digoxin levels (Table 2).

Discussion

In this study, we found that the incidence of digoxin toxicity among a cohort of outpatients was only 1.4 per 100 patient-years of treatment, lower than in previous reports.[4,7] Although metabolic and pharmacologic risk factors were relatively common, few patients even in these "high-risk" groups actually had digoxin toxicity over a one-year period, in part because these risk factors were often transient. For example, digoxin toxicity occurred in only 16.7% of persons with serum digoxin levels above the usual cutoff level of 2.6 nmol per liter. When potentially interacting drugs were added to the digoxin regimen, the clinicians in this study rarely took the precaution of reducing the digoxin dose or measuring serum digoxin concentrations to identify possible drug toxicity.[15] Nevertheless, digoxin toxicity did not occur in any of the nine persons prescribed a new interacting drug.

Studies done 20 or more years ago identified digoxin toxicity in 11% to 30% of hospitalized patients receiving the drug.[1-5] The most recent epidemiologic study of digoxin toxicity among patients admitted to hospitals observed only 27 cases among 563 patients (5%) with CHF, however.[8] Our study suggests that a similar decline has occurred in the incidence of digoxin toxicity among outpatients. Several explanations for this observation are possible. Clinicians may have become more attentive to evidence of possible toxicity, or they may have been more vigilant in monitoring for and correcting metabolic risk factors. Because previous studies do not report the rate of diagnostic monitoring for patients receiving digoxin, we cannot compare the monitoring practices of these VA clinicians with those of other groups of physicians. Whereas some authorities have advocated routinely measuring serum digoxin levels in asymptomatic patients,[16] others have recommended a more sparing use of serum digoxin concentrations[17]—a strategy followed by the clinicians in our study. These clinicians also maintained serum digoxin levels in the lower end of the therapeutic range, which likely reduced the risk of drug toxicity in the event of sudden metabolic changes or the addition of interacting drugs. The rarity of digoxin toxicity in our study was not due to the use of lower digoxin doses, as many earlier studies also reported average digoxin doses of 0.125 to 0.25 mg per day.[1,3,4] Likewise, digoxin toxicity was not prevented by avoiding drug interactions, as most patients prescribed an interacting drug neither were closely monitored with serum levels nor reduced their digoxin dose.

Limitations

Our study must be interpreted with an awareness of its limitations. First, the number of patients from each facility in our study was relatively small, although our overall sample is likely representative of outpatients cared for in rural and urban VA medical centers in the Rocky Mountain West. Second, by selecting patients from pharmacy records, we identified many who had been receiving digoxin for prolonged periods of time, rather than an "inception cohort" of patients newly prescribed the drug. Nevertheless, our findings should be applicable to the heterogeneous group of new and long-term digoxin users commonly seen in outpatient practice. Third, we identified only cases of digoxin toxicity that occurred within the VA system. If some patients in our sample were treated for digoxin toxicity in non-VA settings, we would have underestimated the true incidence of digoxin toxic-

9-57

Case 2:08-md-01968 Document 580-27 Filed 09/08/11 Page 47 of 64 PageID #: 24522

ity. The financial incentives to obtain care in the VA and the location of many of these VA facilities in rural areas with limited access to other sources of care reduce the possibility of ascertainment bias. Fourth, not all patients in this retrospective study had complete laboratory information, which could lead to an underestimation of the true prevalence of risk factors. A higher prevalence of risk factors would further lower their predictive value and would thus strengthen our conclusion that toxicity was uncommon even when risk factors were present. Finally, many of the patients sampled lacked an obvious reason for digoxin use, implying that they may have had less severe heart disease than the patients of an earlier era. Although we found these patients overall to be at low risk of digoxin toxicity, we also identified a large subgroup unlikely to benefit from taking the drug.

Clinician Assessments of Digoxin Toxicity

Our study confirmed our clinical impression and that of others that experienced physicians often differ in their assessment of the likelihood of adverse drug effects in general and digoxin toxicity in particular.[8,10,18] A failure to recognize the potential for clinical disagreement is an important limitation of earlier studies of digoxin toxicity, which commonly used a single reviewer. The diverse clinical backgrounds of our reviewers may explain the discrepancies in their assessments of the likelihood of digoxin toxicity. The lowest proportion of possibly toxic patients was identified by reviewer C, the cardiologist, accustomed to seeing severely ill patients admitted to the hospital with digoxin toxicity, and the highest proportion was identified by reviewer B, the geriatrician, who was trained to seek subtle manifestations of drug toxicity among outpatients with many symptoms. The interrater agreements (κ statistics) in our study are consistent with other published assessments of adverse drug reactions[18] and quality-of-care appraisals.[14]

In recent years, the benefits of digoxin have been reevaluated as new drugs have been introduced for the treatment of CHF and atrial arrhythmia. A recent meta-analysis of clinical trials of digoxin use in patients with CHF has supported the use of digoxin in patients with impaired systolic left ventricular function.[19] Digoxin withdrawal has also recently been shown to precipitate acute CHF in patients concurrently treated with diuretics and angiotensin-converting enzyme inhibitors.[9] Although clinical trials are lacking, some researchers have recently questioned the benefits of digoxin in atrial fibrillation because it may provide insufficient control of ventricular rate during exercise.[20] The ongoing redefinition of the role of digoxin is likely to lead to a reduced use of the drug. Our study in the community setting, along with other recent evidence,[8,9] suggests that this improvement in the precision of digoxin use is concurrent with a decrease in the risk of digoxin toxicity.

Acknowledgment

The Board of Directors of MEDIPRO in VA Medical District 23 provided encouragement and assisted in this study.

REFERENCES

1. Beller GA, Smith TW, Abelmann WH, Haber E, Hood WB: Digitalis intoxication: A prospective study with serum level correlations. N Engl J Med 1971; 284:989-997

2. Howard D, Smith CI, Stewart G, et al: A prospective survey of the incidence of cardiac intoxication with digitalis in patients being admitted to hospital and correlation with serum digoxin levels. Aust NZ J Med 1973; 3:279-284

3. Schapel GJ, Jones TE, Draysey TC, et al: The predictive value of the serum digoxin concentration in the management of hospitalized patients. Ther Drug Monitor 1981; 3:137-142

4. Boman K: Digitalis intoxication in geriatric in-patients. Acta Med Scand 1983; 214:345-351

5. Smith TW, Antman EM, Friedman PL, Blatt CM, Marsh JD: Digitalis glycosides: Mechanisms and manifestations of toxicity. Prog Cardiovasc Dis 1984; 26:413-448

6. Carlson KJ, Lee DC, Goroll AH, Leahy M, Johnson RA: An analysis of physicians' reasons for prescribing long-term digitalis therapy in outpatients. J Chronic Dis 1985; 38:733-739

7. Sueta CA, Carey TS, Burnett CK: Reassessment of indications for digoxin: Are patients being withdrawn? Arch Intern Med 1989; 149:609-612

8. Mahdyoon H, Battilana G, Rosman H, Goldstein S, Gheorghiade M: The evolving pattern of digoxin intoxication: Observations at a large urban hospital from 1980 to 1988. Am Heart J 1990; 120:1189-1194

9. Packer M, Gheorghiade M, Young JB, et al: Withdrawal of digoxin from patients with chronic heart failure treated with angiotensin-converting-enzyme inhibitors. N Engl J Med 1993; 329:1-7

10. Smith TW: Digitalis: Mechanisms of action and clinical use. N Engl J Med 1988; 318:358-365

11. Owen DB: Handbook of Statistical Tables. Reading, Mass. Addison-Wesley, 1962

12. Sackett DL, Haynes RB, Guyatt G, Tugwell P: Clinical Epidemiology. Boston, Mass, Little, Brown, 1991

13. Kramer MS, Feinstein AR: Clinical biostatistics LIV—The biostatistics of concordance. Clin Pharmacol Ther 1981; 29:111-123

14. Goldman RL: The reliability of peer assessments of quality of care. JAMA 1992; 267:958-960

15. Marcus FI: Pharmacokinetic interactions between digoxin and other drugs. J Am Coll Cardiol 1985; 5(suppl):82A-90A

16. Aronow WS: Rationale for routine digoxin levels. JAMA 1990; 264:517-518

17. Spector R, Park GD, Johnson GF, Vesell ES: Therapeutic drug monitoring. Clin Pharmacol Ther 1988; 43:345-353

18. Naranjo CA, Busto U, Sellers EM, et al: A method for estimating the probability of adverse drug reactions. Clin Pharmacol Ther 1981; 30:239-245

19. Jaeschke R, Oxman AD, Guyatt GH: To what extent do congestive heart failure patients in sinus rhythm benefit from digoxin therapy?—A systematic overview and meta-analysis. Am J Med 1990; 88:279-286

20. Beasley R, Smith DA, McHaffie DJ: Exercise heart rates at different serum digoxin concentrations in patients with atrial fibrillation. Br Med J 1985; 290:9-11

PLAINTIFFS' EXHIBITS 012640

Case 2:08-md-01968 Document 580-27 Filed 09/08/11 Page 48 of 64 PageID #: 24523

β-receptor blockers until after they have stabilized for several days to weeks.

Cardiac Glycosides

The cardiac glycosides possess a common molecular motif, a steroid nucleus containing an unsaturated lactone at the C 17 position and one or more glycosidic residues at C 3 (*see* Figure 34–7). *Digoxin* (LANOXIN, LANOXICAPS) and *digitoxin* (CRYSTODIGIN) are both orally active, but only digoxin is in widespread clinical use today. Digitoxin differs from digoxin only by the absence of a hydroxyl group at C 12, resulting in a less hydrophilic compound with altered pharmacokinetics compared to digoxin. The cardiac glycosides have been used for centuries as therapeutic agents. The beneficial effects in heart failure were believed to derive from a positive inotropic effect on failing myocardium and efficacy in controlling the ventricular rate response to atrial fibrillation. However, it is now recognized that the cardiac glycosides also modulate sympathetic nervous system activity, an additional mechanism that may contribute importantly to their efficacy in heart failure.

Mechanisms of Action. ***Inhibition of Na^+, K^+–ATPase.*** All cardiac glycosides are potent and highly selective inhibitors of the active transport of Na^+ and K^+ across cell membranes, by binding to a specific site on the extracytoplasmic face of the α subunit of Na^+, K^+–ATPase, the enzymatic equivalent of the cellular "Na^+ pump." The binding of cardiac glycosides to Na^+, K^+–ATPase and inhibition of the cellular ion pump is reversible and entropically driven. These drugs bind preferentia enzyme following phosphorylation at a β-aspar cytoplasmic face of the α subunit and stabilize formation (known as E_2P). Extracellular K^+ pr phosphorylation of the enzyme as an initial st cation's active translocation into the cytosol, th creasing the affinity of the enzyme for binding ca cosides. This provides one explanation for wh extracellular K^+ reverses some of the toxic effec drugs. The regulation of Na^+, K^+–ATPase by di been reviewed in detail (Eisner and Smith, 199

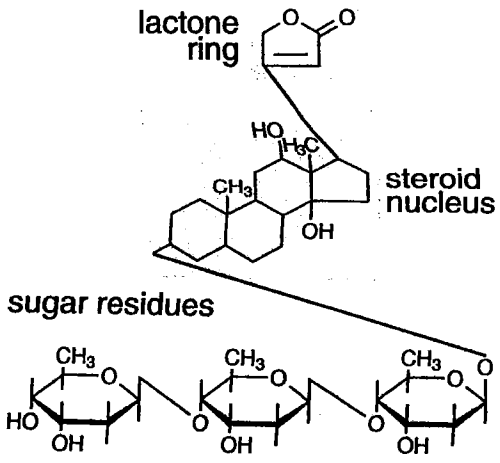


Figure 34–7. Structure of digoxin.

Positive Inotropic Effect. Both Na^+ and Ca^{2-} i cardiac muscle cells during each cycle of depo contraction, and repolarization (Figure 34–8). C enters the cell *via* the L-type Ca^{2+} channel dur larization triggers the release of additional Ca^{2-} cytosol from an intracellular compartment, the s mic reticulum (SR). The greater the amount of Ca^{2+}, the greater the force of contraction. During repolarization and relaxation, Ca^{2+} is pumped the SR by a Ca^{2+}–ATPase and also is removed cell by the Na^+–Ca^{2+} exchanger and by a sar Ca^{2+}–ATPase.

Importantly, the capacity of the exchange trude Ca^{2+} from the cell depends on the intracel concentration. Binding of cardiac glycosides to colemmal Na^+, K^+–ATPase and inhibition of cel pump activity results in a reduction in the rate Na^+ extrusion and a rise in cytosolic Na^+. This in intracellular Na^+ reduces the transmembrane dient driving the extrusion of intracellular Ca^{2-} myocyte repolarization. Hence, some increment taken up into the SR to be made available to the c elements during the subsequent cell depolarizati and contractility of the myocardium is augmente

Electrophysiological Actions. (*see also* Cha Atrial and ventricular muscle and specialized card maker and conduction fibers exhibit differing and sensitivities to cardiac glycosides that are a s of the direct effects of these drugs on cardiac cell indirect, neurally mediated effects. At therapeutic serum or plasma concentrations (*i.e.*, 1.0 to 2. digoxin decreases automaticity and increases ma astolic resting membrane potential predominantl and atrioventricular (AV) nodal tissues, due to an in vagal tone and a decrease in sympathetic ner tem activity. There also is a prolongation of the refractory period and a decrease in conduction v AV nodal tissue. At higher concentrations, this m sinus bradycardia or arrest and/or prolongation of duction or heart block. In addition, cardiac gl

PLAINTIFFS' EXHIBITS 012641

9-59

Case 2:08-md-01968 Document 580-27 Filed 09/08/11 Page 49 of 64 PageID #: 24524

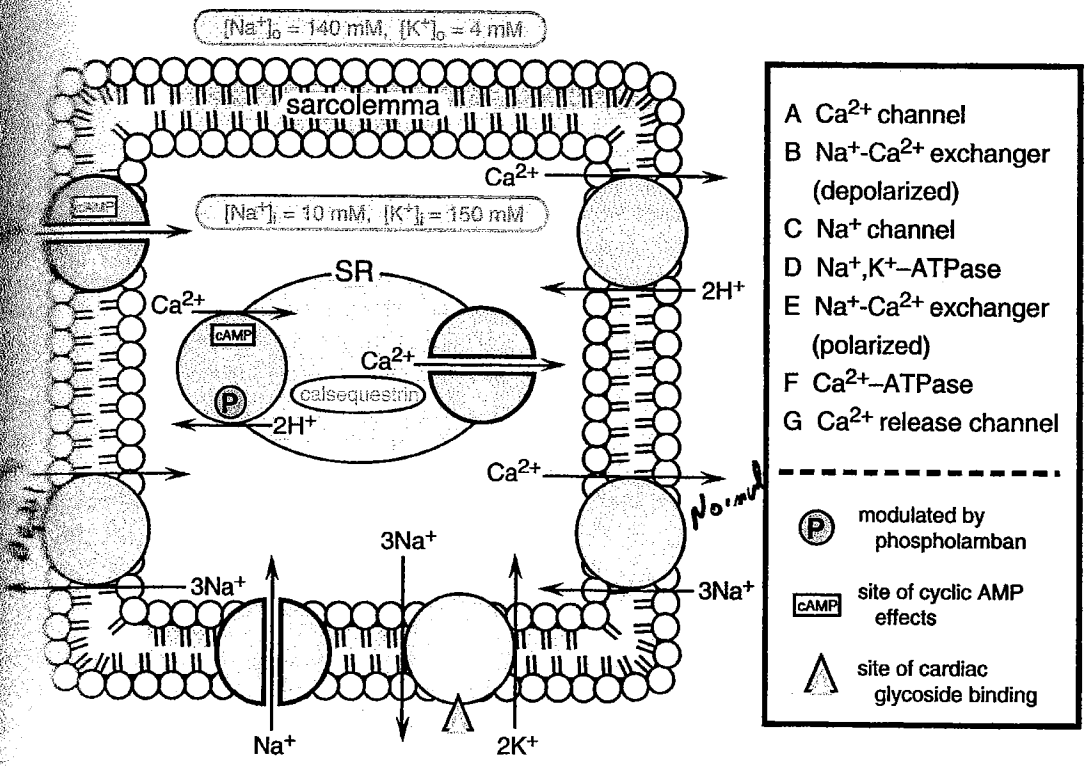


Figure 34–8. Sarcolemmal exchange of Na^+ and Ca^{2+} during cell depolarization and repolarization.

Na^+ and Ca^{2+} ions enter mammalian cardiac muscle cells during each cycle of membrane depolarization, triggering the release, through Ca^{2+} release channels (G), of larger amounts of Ca^{2+} from internal stores in the sarcoplasmic reticulum (SR). The resulting increase in intracellular Ca^{2+} interacts with troponin C and hence is responsible for activating the cross-bridge interactions between actin filaments and myosin cross-bridges that result in sarcomere shortening. The electrochemical gradient for Na^+ across the sarcolemma is maintained by active (*i.e.*, ATP-consuming) transport of Na^+ out of the cell by the sarcolemmal Na^+, K^+–ATPase (D). Na^+ is actively extruded by Na^+, K^+–ATPase, while the bulk of cytosolic Ca^{2+} is pumped back into the SR by a Ca^{2+}–ATPase (F_1), where it is bound by the protein calsequestrin, and the remainder is removed from the cell by either a plasma membrane Ca^{2+}–ATPase or a high capacity Na^+–Ca^{2+} cation exchange protein (B, E). This sarcolemmal membrane protein exchanges 3 Na^+ ions for every Ca^{2+} ion, using the electrochemical potential of Na^+ to drive Ca^{2+} extrusion. Note that the direction of cation transport may reverse briefly during depolarization (B), when the electrical gradient across the sarcolemma is transiently reversed. β-Adrenergic receptor agonists and phosphodiesterase inhibitors, by increasing intracellular cyclic AMP levels, activate protein kinase A, which enhances the contractile state by phosphorylating target proteins, including phospholamban and the α subunit of the L-type Ca^{2+} channel. (Adapted from Smith *et al.*, 1992, with permission.)

concentrations can increase sympathetic nervous activity and directly affect automaticity in cardiac tissues that contribute to the generation of atrial and ventricular arrhythmias. Increased intracellular Ca^{2+} and increased sympathetic tone result in an increase in the spontaneous (phase 4) rate of diastolic depolarization as well as delayed afterdepolarizations that may reach threshold for generation of a propagated action potential. This simultaneous nonuniform increase in automaticity and depression of conduction in His–Purkinje and ventricular muscle fibers predisposes to arrhythmias that may lead to ventricular tachycardia or fibrillation.

Regulation of Sympathetic Nervous System Activity. An increase in sympathetic nervous system activity is one of the physiological responses to a decline in heart function below that required for maintenance of a cardiac output adequate to meet the metabolic demands of body tissues (*i.e.*, heart failure). This is due, in part, to a reduction in

PLAINTIFFS' EXHIBITS 012642

the sensitivity of the arterial baroreflex response to blood pressure, resulting in a decline in tonic baroreflex suppression of CNS-directed sympathetic activity (Ferguson *et al.*, 1989). This desensitization of the normal baroreflex arc also is thought to be responsible in part for the sustained elevation in plasma norepinephrine, renin, and vasopressin levels in heart failure, as well as other indices of systemic neurohumoral activation that are characteristically observed in patients with heart failure. Increased sympathetic nervous system activity initially helps to maintain blood pressure and cardiac output by *increasing* heart rate, contractility, and systemic vascular resistance, and by *decreasing* the excretion of salt and water by the kidneys. However, when sustained chronically, these effects of sympathetic overactivity contribute to the pathophysiology of heart failure and progression of the underlying myocardial disease.

A direct effect of cardiac glycosides on carotid baroreflex responsiveness to changes in carotid sinus pressure has been demonstrated in isolated baroreceptor preparations from animals with experimental heart failure (Wang *et al.*, 1990). In addition, Ferguson *et al.* (1989) demonstrated in patients with moderate to advanced heart failure that infusion of the cardiac glycoside deslanoside increased forearm blood flow and cardiac index and decreased heart rate, while markedly decreasing skeletal muscle sympathetic nerve activity, an indicator of the centrally mediated sympathetic nervous system tone. This was unlikely to have been due predominantly to a direct inotropic effect of the drug, since dobutamine, a sympathomimetic drug that increases cardiac output to a comparable extent, did not affect muscle sympathetic nerve activity in these patients. A reduction in neurohumoral activation could represent an important additional mechanism contributing to the efficacy of cardiac glycosides in the treatment of heart failure.

Pharmacokinetics. The elimination half-life for digoxin is 36 to 48 hours in patients with normal or near-normal renal function. This permits once-a-day dosing for patients with normal or mildly impaired renal function, and near steady-state blood levels are achieved 1 week after initiation of maintenance therapy. Digoxin is excreted for the most part unchanged with a clearance rate that is proportional to the glomerular filtration rate. In patients with congestive heart failure and marginal cardiac reserve, an increase in cardiac output and renal blood flow with vasodilator therapy or sympathomimetic agents may increase renal digoxin clearance, necessitating adjustment of daily maintenance doses. Nevertheless, digoxin is not removed effectively by peritoneal or hemodialysis due to the drug's large (4 to 7 liters/kg) volume of distribution. The principal tissue reservoir is skeletal muscle and not adipose tissue and, thus, dosing should be based on estimated lean body mass. Neonates and infants to... require higher doses of digoxin for ... peutic effect than do older children ... absorption and renal clearance rates ... does cross the placenta, and drug le... umbilical vein blood are similar.

Most digoxin tablets average 70... availability; however, approximately ... population harbors the enteric bacte... *lentum,* which can convert digoxin in... lites, and this may account for some ... resistance to standard doses of oral di... capsules of digoxin (LANOXICAPS) hav... ability than do tablets and require d... a patient is switched from one dosage ... Parenteral digoxin is available for in... tration, and maintenance doses can ... venous injection when oral dosing is ... muscular digoxin administration is e... causes local discomfort, and is not rec... ber of drug interactions (*see* Table 34-... ditions can alter digoxin's pharmacoki... tient's susceptibility to toxic manifesta... Chronic renal failure, for example, ... volume of distribution, necessitating ... tenance dosage of the drug. Electroly... pecially hypokalemia, acid base imba... underlying heart disease also may alte... tibility to toxic manifestations of digo...

Clinical Use of Digoxin in Heart Failu... the turn of the century, there has be... rounding the efficacy of cardiac gly... ment of patients with heart failure whe ... Despite widespread use of digoxin, ... randomized, controlled trials on the s... digoxin had been lacking until the 19...

The PROVED (Prospective Random... ular failure and Efficacy of Digoxin; Tre... RADIANCE (Randomized Assessment ... tion of Angiotensin Converting Enzyme... trials examined the effects of withdrawal ... with stable mild to moderate heart fail... II and III) and systolic ventricular dysfu... ejection fraction <0.35). All patients st... sinus rhythm. Withdrawal of digoxin re... worsening of heart-failure symptoms in ... placebo compared with patients who con... drug. Maximal treadmill exercise tolerance ... icantly in patients withdrawn from digox... continuation of other medical therapies ...

PLAINTIFFS' EXHIBITS 012643

14–4
nteractions with Digoxin

DRUG	MECHANISM	CHANGE IN DIGOXIN BLOOD LEVEL*	SUGGESTED CLINICAL MANAGEMENT
	Pharmacokinetic		
;ramine, –pectin, ·cin, ılazine	Decrease absorption	25% decrease	Give digoxin 8 hours before agent or use solution or liquid-filled capsule form of digoxin
ls	Not known	25% decrease	Temporal dispersion of doses
	Decreases absorption	25% decrease	Temporal dispersion of doses
ıone, ine, quinine mil, ırone	Decrease renal digoxin clearance, volume of distribution, or both	70%–100% increase	Decrease digoxin dose by 50% and monitor serum digoxin levels as necessary
ine	Increases volume of distribution and renal clearance	Variable decreases in digoxin blood levels	Monitor serum digoxin levels
mycin, ızole, cline	Increase digoxin absorption	40%–100% increase	Monitor serum digoxin levels
ol	Increase volume of distribution	30% decrease	Monitor serum digoxin levels
n, ım, ine, ipine	Variable moderate decrease in digoxin clearance and/or volume of distribution	Variable increase in blood levels	Monitor serum digoxin levels
amine	May decrease renal function and, indirectly, digoxin clearance	Variable increase in blood levels	Monitor serum digoxin levels more frequently if renal function impaired
	Pharmacodynamic		
ergic receptor ists, ıol, diltiazem, ine, amide, ol	Decreased sinoatrial (SA) or atrioventricular (AV) junctional conduction or automaticity		Monitor ECG for evidence of SA or AV block
ic diuretics	Decreased serum and tissue K^+, increased automaticity, promotes inhibition of Na^+, K^+–ATPase by digoxin		Monitor ECG for arrhythmias consistent with digoxin toxicity
mimetic drugs	Increased automaticity		Monitor ECG for arrhythmia
ol, diltiazem, ergic receptor ists	Diminished cardiac contractile state		Discontinue or lower dose of Ca^{2+} channel blocker or β-adrenergic receptor antagonist

ation may be monitored as clinically appropriate.
ram; ECG, electrocardiogram; SA, sinoatrial; AV, atrioventricular.

PLAINTIFFS' EXHIBITS 012644

9-62

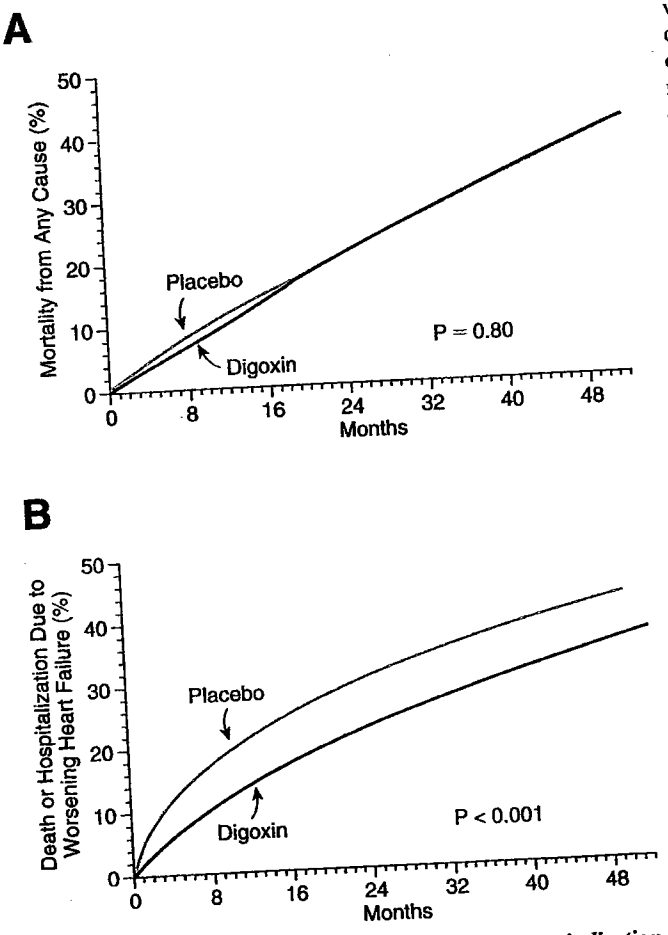


Figure 34–9. Effect of digoxin on survival and hospitalization for heart failure in the Digoxin Investigators Group (DIG) trial.

In the DIG trial, 6800 patients with New York Heart Association class II to III symptoms of heart failure and a left ventricular ejection fraction <0.45 were randomized to digoxin or placebo in addition to standard therapy including ACE inhibitors. There was no difference in mortality between the treatment groups (*Panel A*). However, fewer patients in the digoxin group were hospitalized due to worsening heart failure (*Panel B*). (Adapted from the Digoxin Investigation Group, 1997, with permission.)

The much larger Digoxin Investigators' Group (DIG) trial was designed to detect an effect of digoxin therapy on the survival of patients with heart failure (The Digitalis Investigation Group, 1997). In this randomized, double-blind trial, 6,800 patients with predominantly mild to moderate (NYHA class II to III) heart failure and a left ventricular ejection fraction <0.45 were assigned to receive either digoxin or placebo in addition to standard therapy including ACE inhibitors. A trend was seen toward a decrease in the risk of death attributed to worsening heart failure in the digoxin-treated group was balanced by a small increase in the risk other cardiac causes (presumed to result from overall, no difference in mortality was seen ment groups (*see* Figure 34–9). However, few digoxin group were hospitalized due to worse This benefit was seen at all levels of ejectio greatest in patients with more severe degree Interestingly, in a predefined substudy of pa ejection fraction (*i.e.*, presumed to have diast similar pattern of benefit was seen with digox data, it is recommended that digoxin be re with heart failure who are in atrial fibrillati in sinus rhythm who remain symptomatic de adequate dosages of ACE inhibitors and β- antagonists.

Doses of Digoxin in Clinical Practice a Serum Levels. Using indices of ventricu studies suggest that the greatest increase in parent at serum levels of digoxin around (Kelly and Smith, 1992a). The neurohormon may occur at lower serum levels, between higher serum concentrations than this are further decreases in neurohormonal activati clinical benefit. Furthermore, a subgroup trial (The Digitalis Investigation Group, 1 parent increased risk of death with increas tions, even for values within the traditio Therefore, many authorities advocate main below 1.0 ng/ml.

A common approach for initiating begin at 0.125 to 0.25 mg/day, depending and creatinine clearance, and to measure a week later when a steady-state has been sample should be obtained at least 6 ho digoxin dose. Routine surveillance monit need not be carried out, unless a signi renal function occurs, or a new drug (e. substantially alters digoxin pharmacoki or intravenous loading with digoxin, w rarely necessary as other safer and more for short-term inotropic support.

Digoxin Toxicity. The incidence a toxicity have declined substantially i due in part to the development of the treatment of supraventricular failure, to the increased understan macokinetics, to the monitoring of and to the identification of importa digoxin and many commonly use the recognition of digoxin toxicity consideration in the differential di and/or neurological and gastrointe tients receiving cardiac glycosides

Vigilance for and early rec of impulse formation, conductio

Case 2:08-md-01968 Document 580-27 Filed 09/08/11 Page 53 of 64 PageID #: 24528

Symptoms of Cardiac Glycoside Toxicity

- fatigue, malaise, confusion, dizziness, dreams
- or yellow vision, halos
- *inal*
- nausea, vomiting, abdominal pain
- ventilatory response to hypoxia
- *rhythmias*
- ventricular ectopic arrhythmias
- *disturbances*
- and atrioventricular node conduction nces

Among the more common electrophysiological ions are ectopic beats of AV junctional or ven- gin, first-degree AV block, an excessively slow rate response to atrial fibrillation, or an accel- junctional pacemaker. These often require only adjustment and appropriate monitoring. Sinus bradycardia, sinoatrial arrest or exit block, and second- or third-degree AV conduction delay usually respond to atropine, although temporary ventricular pacing may be necessary. Potassium administration should be considered for patients with evidence of increased AV junctional or ventricular automaticity, even when the serum K^+ is in the normal range, unless high-grade AV block also is present. Lidocaine or phenytoin, which have minimal effects on AV conduction, may be used for the treatment of worsening ventricular arrhythmias that threaten hemodynamic compromise. Electrical cardioversion carries increased risk of inducing severe rhythm disturbances in patients with overt digitalis toxicity, and it should be used with particular caution.

Antidigoxin Immunotherapy. An effective antidote for digoxin or digitoxin toxicity is now available in the form of antidigoxin immunotherapy with purified Fab fragments from ovine antidigoxin antisera (DIGIBIND). A full neutralizing dose of Fab based on either the estimated total dose of drug ingested or the total body digoxin burden (Table 34–6) can be administered intravenously in saline solution over 30 to 60 minutes. For a more comprehensive review of the treatment of digitalis toxicity, *see* Kelly and Smith (1992b).

ation of Dose of Antidigoxin Immunotherapy

lation of the amount of polyclonal antidigoxin Fab antibody fragments to be administered is based on a Fab that is stoichiometrically equivalent to the total body burden of digoxin.

mation of total body digoxin burden (mg):

A. $$\left[\begin{array}{c}\text{Total drug in body (in mg)}\\ \text{(following acute}\\ \text{digoxin ingestion)}\end{array}\right] = \left[\begin{array}{c}\text{Amount}\\ \text{ingested}\\ \text{(in mg)}\end{array}\right] \times \left[\begin{array}{c}\text{Average oral bioavailability}\\ \text{of tablet formulations}\\ \text{(0.8 for digoxin)}\end{array}\right]$$

B. $$\left[\begin{array}{c}\text{Known or suspected}\\ \text{toxicity during chronic}\\ \text{digoxin therapy}\end{array}\right] = \frac{\left[\begin{array}{c}\text{Serum digoxin}\\ \text{concentration}\\ \text{(in ng/ml or } \mu\text{g/l)}\end{array}\right] \times \left[\begin{array}{c}\text{Volume of}\\ \text{distribution}\\ \text{(5.6 liters/kg)}\end{array}\right] \times \left[\begin{array}{c}\text{Weight}\\ \text{(in kg)}\end{array}\right]}{1000}$$

lculation of Fab fragment dose:

$$\left[\begin{array}{c}\text{Dose of}\\ \text{Fab fragments}\\ \text{(in mg)}\end{array}\right] = \frac{\left[\begin{array}{c}\text{Molecular mass of}\\ \text{Fab fragments}\\ \text{(50,000 daltons)}\end{array}\right] \times \left[\begin{array}{c}\text{Total body}\\ \text{digoxin content}\\ \text{(in mg)}\end{array}\right]}{\left[\begin{array}{c}\text{Molecular mass of}\\ \text{digoxin}\\ \text{(781 daltons)}\end{array}\right]}$$

PLAINTIFFS' EXHIBITS 012646

Clinical Toxicology Downloaded from informahealthcare.com by 67.190.80.113 on 04/09/11
For personal use only.

There is great variation among data furnished by different authors. It is a consequence of the use of different clinical, toxicological or forensic criteria; different analytical methods; the variable nature of the samples (some concentrations refer to whole blood, while others refer to plasma/serum); different clinical parameters and pathology. Also, age and sex of the patient; route of administration; presence of multiple drugs; and different times of sampling come into play. Sometimes concentrations of samples obtained several days after poisoning are presented as acute. Lethal concentrations are also described as those found after the absorption of supralethal doses or upon a patient's death following a period of survival with or without therapy. Furthermore, time between death and sampling varies, implicating different postmortem redistribution of medicines.

The objective of this paper is to facilitate the interpretation of analytical results from patients or postmortem samples when there is a suspicion of poisoning with drugs affecting cardiovascular system, blood or hematopoietic organs. The table is intended to give some guidance in interpreting drug levels encountered in clinical, toxicological and forensic cases.

METHODS

All drugs of groups B and C in the Spanish official catalogue of pharmaceutical specialties[2] have been studied. The groups and subgroups are:

Group B: Blood and hematopoietic system:
- B1: Anticoagulants
- B2: Hemostatics
- B3: Antianemic drugs
- B4: Lipid-lowering drugs
- B5: Plasma substitutes
- B6: Fibrinolytic drugs
- B7: Stimulants of hematopoiesis

Group C: Cardiovascular system:
- C1: Heart drugs
- C2: Antihypertensive drugs
- C3: Diuretics
- C4: Cerebral and peripheral vasodilators
- C5: Antihemorrhoidal and antivaricosity agents
- C6: Other cardiovascular drugs
- C7: Beta blocking agents

Previously published data on therapeutic, toxic, and lethal/postmortem concentrations of these two related groups of drugs have been reviewed. The review focuses on values in whole blood, serum/plasma and urine. The recently published tables[3-10] have been studied, in addition to many articles dealing with concentrations of only one drug, as well as data from isolated cases published in selected books[11-14] or included in one computerized clinical information system.[15] Considerations of space impede reference to articles reviewed for individual cases. Thus raw data for some of the drugs cannot be found in the cited references.

We have tried to unify all the values provided by different authors to obtain one approximate concentration applicable in most cases. With some drugs this was very difficult because of the discrepancies among the data. The criteria of conservative selection have been followed, eliminating outliers. In selecting the data, we have also taken variation into account and thus did not accept averages which we considered affected by the wide dispersion of extreme values. We eliminated the latter and chose as most representative the values most often repeated among the different authors. To estimate values, we have also applied our own experience from real poisoning cases analyzed in the National Institute of Toxicology in Seville as well as from calls received at our Toxicological Information Service.

RESULTS

The results can be seen in the Table. The first column gives the generic name of the drug in alphabetical order. The next three columns list therapeutic, toxic, and lethal/postmortem concentrations, respectively. Each of these three columns contains concentrations in whole blood, serum/plasma, and urine, expressed in mg/L.

In this study, the three types of concentrations were defined as follows: *Therapeutic Concentrations*: drug levels without overt toxic symptomatology; *Toxic Concentrations*: the lowest levels producing toxic effects; *Lethal/Postmortem Concentrations*: the lowest levels most frequently found at autopsy.

DISCUSSION

Caution must be used when interpreting these amalgamated, compiled values and comparing them with actual values from a particular case. The actual

RIGHTSLINK

For personal use only.

Phenytoin		3-15	0-4		20	5	50	50	
Piperazine		0.02-0.1			0.5				
Prazosin		0.001-0.03			0.9				
Procainamide *N*-acetylprocainamide*		2.5-8			8-10		20		550†
Promethazine		0.05-0.4			1		2.5	2	
Propafenone in children Norpropafenone*		0.3-1.6			2		7.7† 0.8†		
Propranolol		0.02-0.9			1		2-4	4	1-2
Quinidine	0.3-6	0.3-5	10-100	10	5-15		30	15	
Salicylic acid§ in children		20-250		210	150-300		500	400 300	
Sotalol		0.5-3			5		40	40	400
Spironolactone Canrenone*		0.05-0.5							
Timolol		0.005-0.1							
Tranexamic acid		10-50							
Triamterene		0.01-0.1							
Verapamil Norverapamil*	0.08-0.3	0.05-0.5		0.36†	1		1	2.5	
Warfarin		1-7			10		100		

*Metabolite, shown under the original product, and listed in alphabetical order, where concentrations are given.
†Isolated case.
‡Its active metabolites 1-(5-hydroxyhexyl)-3,7-dimethylxanthin and 1-(3-carboxipropyl)-3,7-dimethylxanthin get concentrations in plasma 5-8 higher than pentoxifylline.
§Accumulated in red cells.

Case 2:08-md-01968 Document 580-27 Filed 09/08/11 Page 55 of 64 PageID #: 24530

PLAINTIFFS' EXHIBITS 012648

RIGHTSLINK

9-66

Chlorthalidone	5-10	0.15-1.4									
Clofibrate		30-60									
Clonidine		0.001-0.002	0.01	0.023							
Desethylenalapril		0.01-0.05									
Diacetolol		0.65-4.5			90						
Diazoxide		10-20	50								
Digitoxin		0.003-0.025	0.03			0.03-0.1					
Digoxin		0-0.002	0.025-0.125	0.003	0.005	0.005					
Dihydroergotamine		0-0.004									
Diltiazem		0.1-0.4	0.8	7	1.3	10-60					
Dipyridamole		0.1-2	4								
Doxazosin		0.01-0.15									
Enalapril (see Desethylenalapril*)											
Ethamsylate		15-20									
Felodipine		0.001-0.008	0.01								
Flecainide		0.2-1	2-3	10	13	55-80					
Flunarizine		0.025-0.2	0.3								
Furosemide		1-6	2-5	25							
Hydralazine		0.05-0.5									
Hydrochlorothiazide		0.074-0.45									
Indomethacin		0.3-3	5								
Iron		380-625	0.5-2	6		17					
in children				2.8							
Isosorbide, dinitrate		0.003-0.018									
Isosorbide, mononitrate		0.1-1									
Isradipine		0-0.002	0.01								
Labetalol		0.025-0.2	0.5								
Lidocaine	1.7-6	0.2-5	6	11	10	6-18					
Monoethylglycinexylidide*											

(continued)

RIGHTS

Clinical Toxicology Downloaded from informahealthcare.com by 67.190.80.113 on 04/09/11
For personal use only.

Table (continued)

Drug	Therapeutic Whole Blood	Therapeutic Serum Plasma	Therapeutic Urine	Toxic Whole Blood	Toxic Serum Plasma	Toxic Urine	Lethal/Postmortem Whole Blood	Lethal/Postmortem Serum Plasma	Lethal/Postmortem Urine
Lisinopril		0.02-0.07			0.5				
Magnesium		16-25 1.3-2 mEq/L 0.5-1 mmol/L	30-300		48.6 4 mEq/L 2 mmol/L			150 15 mEq/L 8 mmol/L	
Methyldigoxin (see digoxin)									
Methyldopa		1-5			7	126		9	1400
Metoprolol	0.025	0.02-0.6			1		10	12	1.6†
Mexiletine		0.5-2			2-4		20	35	370†
Milrinone		0.15-0.25			0.3				
Molsidomine		0.002-0.01							
Monoethylglycinexylidide		0.5-2							
N-acetylacebutolol		1-2.5						100	
N-acetylprocainamide		5-30			40				
Nadolol		0.01-0.25							
Naftidoforyl		<0.5							
Nicardipine	0.02-0.05	0.07-0.1							
Nifedipine	0.02-0.1	0.02-0.1			0.1		0.15		
Nilvadipine		<0.01							
Nimodipine		0.01-0.05							
Nisoldipine		0-0.001							
Nitrendipine		0.01-0.05							
Nitroglycerin		0-0.013							
Norpropafenone		0.07-0.7							
Norverapamil		0.05-0.4			1				
Oxprenolol		0.05-1			2		6	10	
Pentoxifylline‡		0.5-2							
Perazine (see Piperazine)									

Case 2:08-md-01968 Document 580-27 Filed 09/08/11 Page 57 of 64 PageID #: 24532

PLAINTIFFS' EXHIBITS 012650

RIGHTSLINK

9-68

Clinical Toxicology Downloaded from informahealthcare.com by 67.190.80.113 on 04/09/11
For personal use only.

Table

Therapeutic, Toxic, and Lethal Postmortem Concentrations in mg/L of 90 Cardiovascular and Hematopoietic Drugs

Drug	Therapeutic			Toxic			Lethal/Postmortem		
	Whole Blood	Serum Plasma	Urine	Whole Blood	Serum Plasma	Urine	Whole Blood	Serum Plasma	Urine
Acebutolol *N*-acetylacebutolol* Diacetolol*		0.2-1.5					35†		15
Acenocoumarol		0.03-0.1			0.1				
Acetazolamide		10-20		38	25				
Acetyldigoxin (see Digoxin)									
Acetylsalicylic acid Salicylic acid*		20-100			150		500		500
Ajmaline in children	0.01-0.03	0.01-1		0.15			5.5†		
Amiodarone		0.7-2			2.5				
Amiodarone + desethylamiodarone*		1-5			5				
Amrinone		1-4							
Aprindine		0.75-2.5			2-3				
Ascorbic acid		10-34							
Atenolol		0.1-1			2			30	
Bendrofluazide		0.05							
Bendroflumethiazide (see Bendrofluazide)									
Betaxolol		0.005-0.05							
Bisoprolol		0.01-0.1							
Buflomedil		0.2-0.5			25	325†	45	55	
Caffeine		2-10	0-10		15	15	80	80	25
Canrenone		0.05-0.25							
Captopril	0.15-1	0.05-0.5			6		60	60	
Carteolol		0.01-0.1							
Celiprolol		0.05-0.5							

Case 2:08-md-01968 Document 580-27 Filed 09/08/11 Page 58 of 64 PageID #: 24533
PLAINTIFFS' EXHIBITS 012651
9-69

Clinical Toxicology, 35(4), 345-351 (1997)

Therapeutic, Toxic, and Lethal Concentrations in Human Fluids of 90 Drugs Affecting the Cardiovascular and Hematopoietic Systems

M. Rosario Repetto; Manuel Repetto

National Institute of Toxicology, Seville, Spain

For personal use only.

ABSTRACT

Background: Drugs affecting the cardiovascular and hematopoietic systems are frequently involved in poisoning. As a continuation of our previously published study about the concentrations of drugs of abuse, we have compiled published data about these drugs and subjected them to selection and unification on the basis of conservative criteria and our own experience. Results: A compilation of the concentrations of 90 drugs affecting heart, circulation, blood or hematopoietic organs, in whole blood, serum/plasma, and urine, corresponding to therapeutic, toxic or lethal concentrations is given. Although the interpretation of the concentrations is a complex and difficult problem, the presented table can be helpful in interpretation from the actual concentrations of this group of drugs encountered in clinical, toxicological and forensic cases.

INTRODUCTION

As a continuation of our previous paper on the concentrations of 103 drugs of abuse or commonly used addictive medicines,[1] we have now reviewed published data relating to the concentrations of another important group of substances, i.e., drugs affecting heart, circulation, blood and hematopoietic organs. As a result, the concentrations of 90 such drugs in whole blood, serum/plasma, and urine, corresponding to therapeutic, toxic or lethal concentrations have been compiled.

Clinical and forensic interpretation of drug concentrations in biological samples is complex.

Correspondence: Dr. M. Rosario Repetto, Instituto Nacional de Toxicología, PO Box 863, 41080 Sevilla, Spain. Fax: 34/5-437-02-62; Email: kuhn@cica.es

Copyright © 1997 by Marcel Dekker, Inc.

RIGHTSLINK

9-70

data can be affected by many different circumstances and conditions, particularly clinical parameters and other known facts surrounding each case. Age, sex and any pathological characteristics of the affected person must be considered. Factors which influence the toxicokinetics of the pharmaceutical product, such as dose, route of administration, etc., must be taken into account. Therapeutic measures which may have been applied to the patient, the toxicity mechanism of the drug and the concomitant presence of multiple drugs and/or metabolites which could exert interactions must be considered. The length of time after exposure will, for most drugs, influence the blood concentration measured in the actual poisoning.[16] The postmortem diffusion of drugs along a concentration gradient will cause a resultant artificial elevation of drug levels. The origin of postmortem blood samples[17-18] from heart blood or femoral vein may likewise result in different values.

In conclusion, the data listed in the table can be helpful in the analysis of individual cases. Because of the many variables, they are only a rough guide and should not be taken as absolute values. When these values are used to aid interpretation of an actual situation, great caution must be taken to consider all factors particular to that case.

REFERENCES

1. Repetto MR, Repetto M. Habitual, toxic, and lethal concentrations of 103 drugs of abuse in humans. *J Toxicol Clin Toxicol* 1997;35:1-9.
2. *Catálogo de Especialidades Farmacéuticas 1996*. Madrid, Spain: Consejo General de Colegios Oficiales de Farmacéuticos, 1996.
3. Stead AH, Moffat AC. A collection of therapeutic, toxic and fatal blood drug concentrations in man. *Hum Toxicol* 1983;2:437-464.
4. Osselton MD. Toxicological tables: a compendium of pharmacological, therapeutic, and toxicological data on 137 drugs and chemicals in humans. In: *Methodology for Analytical Toxicology*, Vol 3. Sunshine I, ed., Florida: Frankin Book Company, 1985:245-261.
5. Repetto M. El análisis químico-toxicológico. In: *Toxicología Fundamental*, 2nd ed. Barcelona, Spain: Ed Científico-médica; 1988:344-366.
6. DiMaio DJ, DiMaio VJM. Interpretive toxicology: Drug abuse and drug deaths. In: *Forensic Pathology*. New York: Elsevier, 1989:440-487.
7. Meyer FP. Indicative therapeutic and toxic drug concentrations in plasma: a tabulation. *Int J Clin Pharmacol Ther* 1994;32:71-81.
8. Winek CHL. *Drug and Chemical Blood-Level Data 1994*. Pittsburgh: Allegheny County Department Laboratories, 1994.
9. Schulz M, Schmoldt A. [A compilation of therapeutic and toxic plasma drug concentrations]. *Anaesthesist* 1994;43:835-844.
10. Uges DRA. Therapeutic and toxic drug concentrations. *TIAF Bull* 1996;26:S1-S34.
11. Baselt RC. *Analytical Procedures for Therapeutic Drug Monitoring and Emergency Toxicology*, 2nd ed. Massachusetts: PSG Publishing Company, 1987.
12. Baselt RC, Cravey RH. *Disposition of Toxic Drugs and Chemicals in Man*, 3rd ed. Chicago: Year Book Medical Publishers, 1989.
13. Baselt RC, Cravey RH. *Disposition of Toxic Drugs and Chemicals in Man*, 4th ed. Foster City, California: Chemical Toxicology Institute, 1995.
14. Clarke EGC. *Clarke's Isolation and Identification of Drugs in Pharmaceuticals, Body Fluids, and Postmortem Materials*, 2nd ed. Moffat AC, Jackson JV, Moss MS, Widdop B, eds., London: Pharmaceutical Press, 1986.
15. Poisindex®. In: *Micromedex Computerized Clinical Information System®*. Denver, Micromedex, 1996.
16. Ekwall B, Clemedson C, Hallander S, Bondesson I, Crafoord B. Goals, execution and present results of the MEMO programme. *Abstracts of the XVII International Congress of the European Association of Poisons Centres and Clinical Toxicologists*. Marseille, 1996:201.
17. Pounder DJ, Jones GR. Post-mortem drug redistribution—a toxicological nightmare. *Forensic Sci Int* 1990;45:253-263.
18. Prouty RW, Anderson WH. The forensic science of implications of site and temporal influences on postmortem blood-drug concentrations. *J Forensic Sci* 1990;35:243-270.

For personal use only.

RIGHTSLINK

plan(s) for replacing athletes injured or killed in a disaster with others, so that competition may continue.

In the interest of prompt and accurate identification of team members who might be killed in such a disaster, the author recommends that athletic leagues and teams implement concrete plans for the collection and retention in confidence of information and material which would be vital for such identification. This includes, but is not limited to: unique physical, and other identifying features; up-to-date dental records; fingerprints and footprints; and X-rays.

Disturbing as this might be to those involved, all could take some comfort in the knowledge that the likelihood of an accident requiring the use of this material is very small. Yet, although transportation of athletic teams is far safer than it once was, all concerned must be continually vigilant in insuring that it remains so.

References

1. Eckert WG. The Rockne crash. Am J Forensic Med Pathol 1982 Mar;3(1):17–27.
2. Gaddis VH. Mountain ride in a brakeless bus. In: Gaddis VH. Courage in crisis. New York: Hawthorn, 1973;62–6.
3. Lazenby R. The Lakers: A basketball journey. New York: St. Martin's, 1993;123–5.
4. Lowell VW. Airline safety is a myth. Bartholomew House, 1967:216.
5. Marciano. Sports Illustrated 1993 Aug 23;53–6.
6. National Transportation Safety Board Report, NTSB-AAR-71-4.
7. National Transportation Safety Board Report, NTSB-AAR-72-11.
8. National Transportation Safety Board Report, NTSB-AAR-78-10.
9. Frank B. Polish IL-62 explodes: US loses amateur boxing team. In: Frank B, editor. Plane crashes. New York; Bell, 1981;80.
10. Barlay S. The final call. London: Arrow Books Ltd, 1991;195–6.
11. National Safety Council. Accident facts 1994 edition. Itasca (IL), 1994;96.
12. The National Collegiate Athletic Association. NCAA travel handbook: Championship travel committee travel 1994–5. Overland Park (KS), 1994;3–4.
13. Brookes A. Power failure. In: Brookes A. Disaster in the air. Shepperton, Surrey UK: Ian Allan, 1992;124–5.
14. Brookes A. Crew overboard. IN: Brookes A. Disaster in the air. Shepperton, Surrey UK: Ian Allan, 1992;78–86.

Reprints will not be available from the author. Additional information to:
Gordon K. Murphy, M.D.
143 Duffield Road
Derby DE22 1AF, England

Henrik Druid,[1] *M.D. and Per Holmgren,*[2] *B.Sc.*

A Compilation of Fatal and Control Concentrations of Drugs in Postmortem Femoral Blood

REFERENCE: Druid H, Holmgren P. A compilation of fatal and control concentrations of drugs in postmortem femoral blood. J Forensic Sci 1997;42(1):79–87.

ABSTRACT: A compilation of postmortem femoral blood concentrations of drugs is presented. The samples are collected from cases in which the cause of death was: A) certified intoxication by one substance alone, B) certified intoxication by more than one substance and/or alcohol, and C) certified other cause of death without incapacitation due to drugs. The concentrations were compared with blood concentrations detected in suspected drugged drivers (D), and with previously published fatal and therapeutic concentrations. The special features of this compilation are: 1) exclusively femoral blood concentrations are quoted, 2) all analyses are based on samples handled according to a standardized, quality-controlled procedure, 3) two control groups are included, and 4) one-substance-only intoxications are separated from other intoxications. The material is based on a selection of 15,800 samples sent to the Department of Forensic Chemistry in Linköping, Sweden, during 1992 to 1995 from the six forensic pathology units in Sweden, and the list includes 83 drugs. The compilation includes drugs, where previously published data are scarce. Furthermore, the data gathered from cases with other cause of death than intoxication (group C) constitute a new kind of reference information, which probably offers a better estimate of obviously non fatal levels in postmortem blood than any compilation of therapeutic concentrations in living subjects. The possible factors influencing postmortem drug concentrations are discussed.

KEYWORDS: forensic science, forensic toxicology, postmortem, femoral blood, drug concentrations, fatality

The interpretation of postmortem toxicology data is often a crucial factor in the determination of cause of death. The diagnosis of a fatal intoxication must be based on reasonable toxicology results, postmortem findings and circumstances, all taken into account. The toxicological analysis results should never be considered alone, neither should the circumstances or postmortem findings.

Literature on postmortem blood concentrations in fatal intoxications is mainly available in the form of case reports. Some review articles summarize data on therapeutic, toxic, and fatal concentrations of various drugs (1–6), but so far, no compiled information is published about the normal postmortem concentrations of various drugs. Instead, data on therapeutic levels are provided as reference values for the range of normal serum or plasma concentrations. Because, however, neither serum nor plasma, but whole blood is used for postmortem toxicological analyses, clinical information about therapeutic concentrations is not always applicable, because of variations in erythrocyte binding between drugs.

Whereas the concentrations of some substances seem to remain essentially unchanged after death, others may increase or decrease postmortem (7–17). Furthermore, several studies have disclosed postmortem redistribution of various drugs (7,9,11–15,18–21) causing differences in concentrations between sampling sites. Unfortunately, the sampling site is not always stated in published material, making interpretation of the concentrations difficult. In addition, many reports lack information about method of collection, storage conditions, addition of preservatives, and presence of other drugs or alcohol. Therefore, present knowledge of therapeutic, toxic, and fatal levels probably contains several pitfalls.

Complaints about lack of correspondence between values presented in different compilations have recently led to debate (22,23). Discrepancies of this type may, of course, be explained by the fact that the authors tend to vary in their evaluation of published and own material. The major disadvantage with all compilations of published toxicology data is, however, the lack of standardized material. Values may be based on heart blood or peripheral blood, or both. In many reports, the sampling site is not even mentioned. For a number of drugs, the sampling site may considerably affect the blood concentration due to postmortem redistribution (7,9,11,12,15,18–21).

To overcome these problems, and because knowledge about the possible overlap between non fatal and fatal concentrations of various drugs must be generally available, consistent sampling and analyses of specimens from deceased control cases is necessary. Thus, we have compiled a list of postmortem drug concentrations, based on Swedish postmortem toxicology data obtained under standardized conditions regarding sample site, sampling technique, analytical methods, and sample storage and treatment.

Material and Methods

Material

During the 1992–1995 period, a total of 15,800 blood samples were collected at medicolegal autopsies performed in Sweden. All toxicology results were recorded in the forensic toxicology database, enabling rapid retrieval of all positive findings (24). In accordance with instructions from the National Board of Forensic Medicine (the authority responsible for all forensic pathology, toxicology, serology, and psychiatry activities in Sweden) all forensic pathology units in the country use the same standardized routines for sample collection and handling (25).

[1]Forensic pathologist, Department of Forensic Medicine, University Hospital, Linköping, Sweden.
[2]Chemist, Department of Forensic Chemistry, National Board of Forensic Medicine, University Hospital, Linköping, Sweden.

Received 20 Feb. 1996; and in revised form 19 April 1996; accepted 26 April 1996.

Case 2:08-md-01968 Document 580-27 Filed 09/08/11 Page 61 of 64 PageID #: 24536

9-72

Case 2:08-md-01968 Document 580-27 Filed 09/08/11 Page 62 of 64 PageID #: 24537

Density of Blood = 1.060 mg/ml

Collection of Samples

At the autopsy, femoral blood (when available), is collected in a 20-mL plastic tube. The blood is collected by cutting off the iliac veins (avoiding the arteries) using a clean knife and pressing the blood in the popliteal and femoral veins into the tube. The blood from both sides is pooled. Potassium fluoride is added to a concentration of 1% using an automatic pipette. Care is taken to avoid blood from the lower vena cava; the blood in the upper portion of the iliac veins is pressed upwards before cutting.

Handling of Samples

All samples are labeled with case number, name, and civic registration number of the deceased, and with sample site. These data are verified by comparison with the label attached to the body, with the data in the police report, and with other documents. After addition of potassium fluoride, the samples are shaken carefully to achieve an even distribution of the additive and stored at 4°C until analyzed (except during transportation). The identity of each sample is checked on several occasions at the forensic pathology unit and at the forensic toxicology laboratory, in accordance with standardized routines.

Other Samples

Urine and vitreous humor are also routinely collected. For alcohol analysis, a separate portion of femoral blood and urine is collected in prefluoridized, 5-mL plastic tubes. Supplementary samples, such as heart blood, liver, skeletal muscle, liquor, and stomach content are collected if standard samples are lacking, or if this is considered necessary for obtaining additional information.

Selection and Classification

Information about the cases was obtained from the forensic toxicology and forensic pathology databases (24). The rough selection was based on the ICD-9 codes linked with the cause-of-death diagnoses made by the responsible pathologist.

We decided to exclude illicit drugs from this compilation because the interpretation of these substances requires a different approach than that used for this study, particularly due to the extensive intravenous usage and significant interindividual differences in tolerance.

Furthermore, cases in which intravenous administration of other drugs could be suspected were excluded as far as possible. For most drugs, oral intake was either certain or highly probable, but for ketamin, lidocain, mepivacain, pethidine, and tiopental, intravenous administration was likely.

The remaining cases were primarily classified as follows:

Intoxications—Cases in which the pathologist had stated "intoxication by drug(s)" as the immediate cause of death. Manner of death was not taken into account. The continued computer-assisted selection comprised the following exclusion criteria: Lack of femoral blood, hypothermia, massive aspiration, drowning, concomitant gas poisoning, and severe diseases. Resuscitation was not an exclusion criteria, but cases subject to more intensive health care intervention were eliminated. The remaining cases constituted the A and B groups described below.

Controls—Cases in which the pathologist had diagnosed as hanging, shooting, self-stabbing, and suicide by other methods, but not drowning or intoxication. To this category we also added a number of cases with trauma diagnoses due to accidents.

The continued computer-assisted selection of these cases comprised the following exclusion criteria: Lack of femoral blood, injuries to thorax or abdomen, and health care intervention. In addition, all cases in which the circumstances left unanswered the question about possible impairment by drugs were excluded. The remaining cases constituted group C, described in detail below.

Further Selection and Considerations

All toxicology findings in the cases selected in the intoxication group were further subject to manual interpretation, independently by the authors and, finally discussed in detail. Each case was scrutinized regarding the importance of every substance present and special attention was paid to the concentration of alcohols (if present). Clean cases, i.e., cases with presence of one substance alone, constituted group A. In cases with high concentrations of two or more substances, both concentrations were classified as group B values. Thus, the same case may contribute to the B-group values of more than one substance.

Unexpectedly high or low concentrations were examined after the preliminary classification, autopsy protocols; police reports, and all other original documents from the A and B cases were perused. Accordingly, the original files of control cases with unexpectedly high concentrations were also checked.

Decomposition was not an exclusion criteria. Some degree of decomposition was present in 16% of the cases. Nine substances from different groups of drugs were studied with special reference to the influence of decomposition.

Special attention was paid to the concentration of alcohols (if present). For most drugs, a concentration of ethanol below 0.1% was accepted in A cases. We considered the possibility of classifying the C cases similarly, i.e., to separate cases with a given substance as the only finding from cases in which additional substances, including alcohol, were detected. Our conclusion was, however, that this was likely to cause confusion and complicate the interpretation of the list. This alternative was thus discarded, and a control case may therefore contribute to the C-group values of several substances.

In summary, the finally included cases were classified as follows: Group A: Certified deaths by intoxication including only clean cases, i.e., in which influence of alcohol or other substances and other contributory factors could be ruled out. Group B: Certified deaths by intoxication in which more than one substance and/or significant alcohol concentrations were found. Group C: Certified other cause of death, in which the circumstances excludes the possibility of incapacitation by drugs. In addition, a second control group was established: Group D: Suspected-drugged drivers (blood samples collected 1992–1994 from living subjects and analyzed at the Department of Forensic Chemistry in Linköping).

Following the selection procedure, statistical processing was performed using Statistica™ from StatSoft Inc, Tusla, Oklahoma, USA. Comparisons between means of the different decomposition groups were made by using Student's *t*-test. A *P*-value of <0.05 was considered significant. Percentiles were calculated if subgroups included at least 10 cases. Quartiles were calculated when subgroups contained four to nine cases. The median value was calculated for all subgroups.

Analytical Methods

In all cases, the following analytical methods were used. Ethanol and other alcohols were analyzed by head-space gas-chromatography. Analyses were always performed in two different specimens, normally femoral blood and urine or vitreous humor. Salicylate and oxazepam were analyzed using HPLC, and trichloro-ethanol was analyzed using a spectrophotometric method. All other drugs were analyzed by gas-chromatography utilizing HP 5880A gas chromatographs equipped with HP 7673A autoinjectors and NP detectors.

Two different extraction methods were used according to the following procedures. An alkaline extract was made by extracting 1.0 g of femoral blood with 0.4-mL butyl-acetate after the addition of 0.3-mL 1 M trisbuffer, pH 11, and 0.03-mL internal standard (0.05 mg cyclizine and 0.10 mg mesoridazine per mL). After extraction for 10 min and subsequent centrifugation, an aliquot was injected in split-mode into a DB-5 (15 m by 0.25 mm ID, 0.25 μm thickness). The injector temperature was 250°C, and the temperature was increased in increments from 200 to 300°C. The total run time was about 17 min.

A neutral extract was made using 1.0 g of femoral blood, 0.5 mL 0.5 M phosphate buffer pH 7.0, 0.05 mL internal standard (0.1 mg allobarbital and 0.01 mg prazepam per mL), and extraction with 0.5-mL butyl-acetate for 10 min. After centrifugation, an aliquot was injected in split-mode into the column. The injector temperature was 250°C and the column used was a SE-54 (25 m by 0.31 mm ID, 0.17 μm thickness). The temperature was increased in increments from 150°C to a final temperature of 300°C. The total run time was about 20 min.

Standard curves used for the quantitation of the drugs were made by adding known amounts of each drug to drug-free blood and plotting the area response ratio for drug and internal standard versus the concentration of the drug. For each drug investigated, a linear correlation was achieved. In each run, several internal controls were used to achieve high quality and similar results over time. The laboratory participates in international quality assurance programs.

Results

Table 1 shows the femoral blood concentrations of the drugs studied, distributed according to the groups as described in "Selection and classification." The data are given in μg/g blood. The molecular weight of each substance is also shown. The parent substances are sorted alphabetically, with the metabolite (if presented) directly following the parent drug. Drugs with fewer than five cases in groups A, B, and C together are not listed.

Because of the significant postmortem transformation of the benzodiazepines clonazepam, flunitrazepam, and nitrazepam into their 7-amino metabolites, the concentrations of the parent drug and metabolite are added in the table.

Because nortriptyline is marketed as such in Sweden, it was considered to be the parent drug when found alone. However, when occurring together with amitriptyline, we counted it as the metabolite of amitriptyline, despite the (unlikely) possibility of ingestion of both nortriptyline and amitriptyline. Nortriptyline values are therefore presented twice in the table.

Desipramine as such is not marketed in Sweden. It occurs in the toxicology material as the result of the breakdown of either imipramine or lofepramine. Whereas imipramine is easily detected, lofepramine may escape detection. Thus, because the origin of desipramine often is unknown, it is presented separately.

TABLE 1—*Femoral blood concentrations of 83 substances. Group A = fatal intoxication with the substance exclusively. Group B = fatal intoxication with the substance in combination with other drugs and/or alcohol. Group C = other cause of death without incapacitation due to drugs. Group D = Concentrations in whole blood from suspected-drugged drivers. In groups A to C, concentrations refer to femoral blood. LOW = lower percentile (N > 9), lower quartile (N = 4–9), or minimum value (N < 4). HIGH = upper percentile (N > 9), upper quartile (N = 4–9), or maximum value (N < 4). FLU = flunitrazepam. CLO = clonazepam. NIT = nitrazepam. All values are given in μg/g. The numbers beneath the drug names refer to the molecular weights, enabling calculation of molarities. Substance names are given according to Clark's isolation and identification of drugs (3). For some drugs, common synonyms are displayed in brackets.*

Substance	Case Type	N	Low	Median	High
Acetaminophen	A	0			
(*Paracetamol*)	B	139	90	170	320
151.2	C	168	1.0	5.0	13
	D	67	0.9	4.0	22
Alimemazine	A	11	1.0	1.6	3.2
(*Trimeprazine*)	B	9	0.5	0.9	1.2
298.4	C	15	0.1	0.1	0.4
	D	3	0.06	0.1	0.1
Desmethylalimemazine	A	11	0.2	0.7	1.3
284.4	B	8	0.2	0.3	0.5
	C	9	0.1	0.2	0.2
	D	2	0.07	0.14	0.2
Alprazolam	A	0			
308.8	B	5	0.3	0.3	0.4
	C	6	0.02	0.05	0.05
	D	22	0.02	0.05	0.18
Amitriptyline	A	49	1.2	3.2	14
277.4	B	39	0.5	1.4	6.0
	C	29	0.1	0.2	0.6
	D	7	0.05	0.09	0.1
Nortriptyline, metabolite	A	46	0.2	0.8	3.1
263.4	B	33	0.1	0.3	1.2
	C	23	0.1	0.1	0.4
	D	4	0.08	0.09	0.3
Biperiden	A	0			
311.5	B	4	0.25	0.29	0.66
	C	2	0.02	0.04	0.06
	D	0			
Caffeine	A	0			
194.2	B	9	21	30	32
	C	7	12	17	30
	D	0			
Carbamazepine	A	7	35	45	70
236.3	B	9	10	14	19
	C	56	0.5	4.5	10
	D	30	0.9	4.0	8.3
Carisoprodol	A	14	9.3	25.5	40
260.3	B	16	5.4	11.5	37
	C	7	0.4	0.7	1.8
	D	31	0.4	2.8	8.4
Chlordiazepoxide	A	1		4.4	
299.8	B	4	2.7	2.9	3.0
	C	12	0.1	0.2	1.3
	D	12	0.3	1.1	6.0
Chlormezanone	A	1		18	
273.7	B	7	11	14	16
	C	6	0.3	1.3	6.3
	D	17	0.4	1.5	14
Chloroquine	A	6	1.3	23.5	35.5
319.9	B	3	0.4	1.2	16
	C	9	0.2	0.9	1.7
	D	0			
Chlorpromazine	A	1		6.7	
318.9	B	2	0.8	1.6	2.4
	C	4	0.1	0.1	0.2
	D	0			

PLAINTIFFS EXHIBITS 01265

9-73

TABLE 1—*Continued*

Substance	Case Type	N	Low	Median	High
Desmethylchlorpromazine 304.9	A	1		0.1	
	B	0			
	C	0			
	D	0			
Chlorprothixene 315.9	A	2	1.6	1.6	1.7
	B	2	0.6	3.8	7.0
	C	1		0.2	
	D	0			
Chlorzoxazone 169.6	A	0			
	B	13	8.0	11	28
	C	8	0.6	1.1	4.6
	D	6	0.3	2.2	3.1
Citalopram 324.4	A	8	3.4	7.0	10.5
	B	13	0.7	1.1	4.7
	C	71	0.1	0.6	1.1
	D	22	0.06	0.15	0.4
Desmethylcitalopram 310.4	A	8	0.1	0.3	0.7
	B	13	0.1	0.1	0.6
	C	43	0.1	0.2	0.3
	D	9	0.05	0.08	0.1
Clomipramine 314.9	A	9	1.6	1.9	2.4
	B	37	0.6	1.1	5.0
	C	61	0.1	0.2	0.4
	D	17	0.02	0.08	0.4
Desmethylclomipramine 300.9	A	9	0.8	1.4	2.0
	B	37	0.2	0.7	4.9
	C	46	0.1	0.2	0.7
	D	12	0.06	0.3	0.5
7-amino-clonazepam 285.7	A	0			
	+CLO B	4	0.3	0.6	1.0
	C	6	0.06	0.13	0.18
	D	15	0.02	0.04	0.28
Clozapine 326.8	A	2	1.2	3.2	5.2
	B	0			
	C	6	0.1	0.6	1.1
	D	0			
Cocaine 299.4	A	1		0.6	
	B	25	0.5	1.1	2.6
	C	20	0.02	0.05	0.4
	D	44	0.005	0.04	0.4
Codeine 299.4	A	0			
	B	4	0.9	1.2	1.5
	C	11	0.1	0.2	0.8
	D	2	0.05	0.06	0.06
Diazepam 284.7	A	0			
	B	0			
	C	90	0.1	0.1	0.3
	D	275	0.1	0.2	0.8
N-desmethyldiazepam *(nordazepam)* 270.7	A	0			
	B	0			
	C	89	0.1	0.1	0.3
	D	251	0.1	0.2	0.7
Diltiazem 414.5	A	0			
	B	0			
	C	13	0.1	0.2	0.4
	D	0			
Dixyrazine 427.6	A	2	5.5	7.5	9.4
	B	12	0.8	2.0	9.0
	C	3	0.2	0.2	0.5
	D	1		0.4	
Ephedrine 174.2	A	0			
	B	0			
	C	10	0.1	0.2	0.6
	D	16	0.05	0.3	2.0
Ethylmorphine 313.4	A	0			
	B	5	0.2	0.4	0.9
	C	0			
	D	7	0.01	0.01	0.02
Flunitrazepam 313.3	A	0			
	B	0			

TABLE 1—*Continued*

Substance	Case Type	N	Low	Median	High
	C	5	0.02	0.03	0.05
	D	130	0.01	0.01	0.05
7-amino-flunitrazepam 283.3	+ FLU A	44	0.16	0.31	0.64
	+ FLU B	139	0.06	0.14	0.43
	C	73	0.01	0.02	0.12
	D	143	0.01	0.02	0.06
Fluvoxamine 318.4	A	4	3.4	5.0	10.7
	B	10	1.2	3.5	8.1
	C	9	0.2	0.5	0.7
	D	1		0.3	
Hydroxyzine 374.9	A	1		2.5	
	B	8	0.9	1.3	1.5
	C	6	0.1	0.2	0.4
	D	5	0.05	0.2	1.0
Imipramine 280.4	A	0			
	B	4	1.2	1.4	2.8
	C	4	0.1	0.2	0.5
	D	1		0.1	
Ketamine 237.7	A	0			
	B	0			
	C	43	0.3	1.0	3.4
	D	5	0.1	1.0	1.0
Ketobemidone 247.3	A	3	0.2	0.3	0.6
	B	5	0.3	0.4	0.5
	C	5	0.03	0.05	0.12
	D	2	0.05	0.06	0.07
Lidocaine 234.3	A	0			
	B	6	1.0	2.1	2.1
	C	113	0.1	0.2	1.2
	D	5	0.06	0.2	0.3
Maprotiline 277.4	A	8	2.7	3.5	5.8
	B	6	1.0	2.7	3.6
	C	11	0.1	0.3	0.9
	D	0			
Melperone 263.4	A	5	1.0	3.7	3.8
	B	7	3.0	5.9	11
	C	6	0.1	0.2	0.2
	D	0			
Dihydromelperone 265.3	A	5	0.6	1.1	1.2
	B	7	1.3	2.6	3.0
	C	6	0.1	0.1	0.2
	D	0			
Mepivacaine 246.4	A	0			
	B	0			
	C	21	0.1	0.3	1.3
	D	8	0.2	0.3	0.3
Meprobamate 218.3	A	3	130	245	260
	B	16	22	31	73
	C	5	2.8	3.5	4.6
	D	34	3.7	12.5	37
Methadone 309.5	A	6	0.5	0.8	1.1
	B	0			
	C	3	0.1	0.1	0.3
	D	24	0.05	0.1	0.2
Methotrimeprazine *(Levomepromazine)* 328.5	A	5	0.8	2.3	3.2
	B	26	0.5	0.9	3.5
	C	15	0.1	0.1	1.7
	D	4	0.04	0.07	0.1
Desmethylmethotrimeprazine 314.5	A	5	0.4	2.3	4.5
	B	21	0.2	0.5	1.7
	C	11	0.1	0.2	1.0
	D	3	0.08	0.1	0.2
Mianserin 264.4	A	3	1.6	2.8	13
	B	8	0.7	0.9	1.3
	C	50	0.03	0.08	0.2
	D	3	0.01	0.02	0.04
Desmethylmianserin 250.4	A	3	1.4	1.5	5.1
	B	5	0.1	0.3	0.4
	C	26	0.03	0.1	0.2
	D	0			

TABLE 1—*Continued*

Substance	Case Type	N	Low	Median	High
Midazolam 325.8	A	0			
	B	0			
	C	21	0.03	0.05	0.4
	D	0			
Moclobemide 268.7	A	0			
	B	10	1.9	4.4	21
	C	17	0.2	0.6	2.1
	D	2	0.3	0.4	0.5
Oxomoclobemide 282.7	A	0			
	B	10	0.3	0.9	2.4
	C	18	0.2	0.5	0.8
	D	1		0.5	
Nitrazepam 281.3	A	0			
	B	0			
	C	12	0.01	0.02	0.03
	D	85	0.02	0.05	0.19
7-amino-nitrazepam 251.3	+ NIT A	16	0.5	1.2	2.8
	+ NIT B	57	0.2	0.8	1.8
	C	90	0.04	0.08	0.2
	D	83	0.02	0.05	0.2
Nortriptyline 263.4	A	5	1.6	3.8	4.0
	B	8	1.9	2.3	3.3
	C	0			
	D	0			
Orphenadrine 269.4	A	18	5.3	15	145
	B	10	4.6	8.9	40
	C	17	0.1	0.3	1.6
	D	9	0.1	0.2	0.2
Oxazepam 286.7	A	2	4.4	5.3	6.1
	B	5	2.3	3.6	3.7
	C	20	0.1	0.3	0.7
	D	76	0.2	0.5	1.4
Paroxetine 329.4	A	0			
	B	6	0.7	4.5	4.6
	C	15	0.09	0.3	0.5
	D	1		0.09	
Pentobarbital 226.3	A	2	10	34	58
	B	1		10	
	C	11	0.1	0.4	1.4
	D	10	0.1	1.2	4.9
Pethidine 247.3	A	0			
	B	0			
	C	8	0.2	0.3	0.5
	D	9	0.1	0.2	0.3
Phenazone 188.2	A	0			
	B	24	13	31.5	100
	C	10	2.1	6.3	28
	D	22	0.8	2.5	11
Phenobarbital 232.2	A	9	55	75	114
	B	4	25.5	36.5	47
	C	4	2.5	7.0	13
	D	11	1.0	17	90
Phenylpropanolamine 151.2	A	0			
	B	0			
	C	5	0.06	0.2	0.3
	D	4	0.09	0.1	0.2
Phenytoin 252.3	A	1		43	
	B	1		80	
	C	14	1.0	5.0	14
	D	5	3.0	11	11.5
Promethazine 284.4	A	3	1.8	2.4	5.4
	B	9	0.6	5.2	11.8
	C	11	0.1	0.1	0.3
	D	6	0.03	0.1	0.1
Desmethylpromethazine 270.4	A	3	0.3	1.4	1.8
	B	9	0.1	0.3	1.6
	C	7	0.1	0.1	0.2
	D	1		0.07	
Propiomazine 340.5	A	24	0.1	0.9	5.4
	B	85	0.08	0.3	1.9

TABLE 1—*Continued*

Substance	Case Type	N	Low	Median	High
	C	14	0.03	0.05	0.4
	D	4	0.03	0.04	0.08
Dihydropropiomazine 342.5	A	24	0.9	1.9	5.8
	B	85	0.3	0.7	2.0
	C	44	0.03	0.08	0.2
	D	3	0.04	0.09	0.1
Propoxyphene *(dextropropoxyphene)* 339.5	A	72	1.3	2.8	8.1
	B	223	0.9	2.0	5.9
	C	89	0.1	0.2	0.8
	D	31	0.04	0.1	0.4
Propranolol 259.3	A	7	4.6	11	13
	B	5	3.9	5.8	5.9
	C	2	0.1	1.1	2.0
	D	3	0.1	0.1	1.9
Remoxipride 371.3	A	11	41	65	150
	B	10	3.6	23.5	73
	C	15	0.7	1.6	4.1
	D	4	0.07	0.2	0.1
Salicylate 138.1	A	1		940	
	B	3	150	158	230
	C	5	15	36	39
	D	0			
Theophylline 180.2	A	4	85	110	140
	B	7	20	62	100
	C	27	1.0	4.0	10
	D	3	0.6	2.0	4.0
Thiopental 242.3	A	0			
	B	0			
	C	36	0.1	0.5	2.1
	D	5	1.2	4.0	4.1
Thioridazine 370.6	A	5	2.4	3.3	3.5
	B	14	1.0	1.8	3.8
	C	19	0.1	0.3	0.7
	D	9	0.1	0.2	0.4
Trichloroethanol 149.4	A	16	60	125	390
	B	6	27	98	109
	C	0			
	D	0			
Trimethoprim 290.3	A	0			
	B	0			
	C	6	0.4	2.2	5.9
	D	0			
Trimipramine 294.4	A	10	1.7	3.5	8.2
	B	17	0.7	1.9	15
	C	8	0.4	0.5	0.8
	D	2	0.05	0.1	0.2
Desmethyltrimipramine 280.4	A	10	0.3	0.8	2.5
	B	16	0.1	0.5	1.2
	C	7	0.1	0.2	0.5
	D	0			
Verapamil 454.6	A	1		3.9	
	B	5	1.6	1.9	3.6
	C	20	0.1	0.2	1.0
	D	0			
Norverapamil 440.6	A	1		1.3	
	B	5	0.1	0.8	0.8
	C	17	0.1	0.1	0.3
	D	0			
Vinbarbital 224.3	A	2	17	18	19
	B	4	12.1	15.1	18.5
	C	0			
	D	0			
Zolpidem 307.4	A	9	0.9	1.3	1.3
	B	3	0.08	0.1	0.12
	C	9	0.13	0.16	0.23
	D	4	0.6	0.7	1.8
Zopiclone 388.5	A	16	0.4	1.2	2.3
	B	10	0.06	0.08	0.4
	C	26	0.03	0.11	0.4
	D				

PLAINTIFF'S EXHIBIT 02668

Case 2:08-md-01968 Document 580-27 Filed 09/08/11 Page 63 of 64 PageID #: 24538

Table 2 shows the influence of decomposition on concentrations of nine selected drugs. The degree of decompesition is based on the pathologist's note on the toxicology request, in which three alternatives are available: None, moderate, or severe. Cases with moderate or severe decomposition were treated as one group and compared with nondecomposed cases. Eight out of nine substances investigated were not affected by decomposition. Flunitrazepam (i.e., parent drug and 7-aminoflunitrazepam together), however, showed significantly higher levels in the decomposed than in the non decomposed group.

Table 3 displays the number of cases with a positive finding of the 100 most commonly detected substances, arranged according to number of cases. Ethanol and carbon monoxide are omitted, though commonly encountered.

Figures 1a–c shows graphically the levels of citalopram, carisoprodol, and methotrimeprazine. The line plots illustrates the differences in overlap between the A, B, C and D groups of these substances.

Discussion

The data presented in Table 1 are based on consistent sampling of femoral vein blood (when available) from all autopsy cases, not only from suspected intoxications. The primary selection is based on the diagnoses (immediate cause of death) made by the responsible forensic pathologist; not by the present authors. The adjustments made by the authors are few and mainly limited to exclusion of cases according to the specifications described above.

Compared with previously published compilations (1–6), the ranges for fatal concentrations are similar for most drugs presented in Table 1. Naturally, there are some differences, which may be explained by e.g., the population studied (the distribution of age, sex, and race) sampling site, average postmortem interval, and analytical methods.

It is hardly meaningful to compare our control levels with therapeutic levels, because we do not know whether the concentrations in the C group represents therapeutic, subtherapeutic, or toxic levels. What we do know is that the deceased in this group had some degree of capacity to perform complex tasks, and yet displayed the blood levels presented.

The same applies to the cases in group D. These suspected drugged drivers were either caught in routine police controls or stopped because of deviant driving behavior. In common with group C, they all had the capacity to perform complex tasks, such as driving a car (although not always safely). As in group C, the concentrations in group D may represent therapeutic, subtherapeutic, or toxic levels. The other important similarity between groups C and D is that the analyses were made on whole blood, whereas therapeutic levels reported in the literature almost invariably refer to concentrations in plasma or serum. The advantage of including groups C and D as controls to groups A and B is illustrated in Fig(s). 1a–c, where the overlap between the groups is evident. This overlap implies that concentrations within this range in some cases may be due to intoxication and explain the death, whereas in other cases, the deceased may not have been significantly incapacitated.

Some work has been conducted regarding the influence of time after death on postmortem drug levels (7–9,11–17,20,21,26). It seems obvious that some drugs may accumulate in different organs, such as the lungs and the liver, and a subsequent diffusion from these sources to the blood presumably takes place for several substances postmortem, causing a rise in blood concentration with time (7–9,11–15). For some substances, postmortem degradation may be a more important phenomenon (10,16,17,27, unpublished observations). In conclusion, current knowledge concerning influence of time after death on drug concentration changes should be borne in mind when using the data presented in Table 1.

In 16% of the total material, some degree of decomposition was reported by the pathologist in charge. However, eight out of nine studied substances did not show significant changes due to decomposition. Therefore, we decided not to exclude cases because of decomposition. For natural reasons, severely decomposed cases are underrepresented, because femoral blood often is lacking under such circumstances. These cases comprise considerable diagnostic problems in many respects, and interpretation of toxicology data

TABLE 2—*Effect of decomposition on blood drug levels. Mean ± SD. No = no significant decomposition changes of the body. Yes = moderate or severe degree of decomposition of the body.*

Substance	No	Yes
Alimemazine (*Trimeprazine*)	1.00 ± 7.39 (183)	1.06 ± 3.40 (41)
Amitriptyline	1.96 ± 4.32 (244)	1.28 ± 2.82 (52)
7-amino-nitrazepam	0.32 ± 0.48 (535)	0.32 ± 0.39 (138)
Citalopram	0.87 ± 2.91 (349)	0.91 ± 1.40 (52)
Clomipramine	0.82 ± 1.63 (232)	0.91 ± 1.03 (39)
Dihydropropiomazine	0.73 ± 4.75 (457)	0.73 ± 1.64 (108)
Propoxyphene (*Dextropropoxyphene*)	1.80 ± 5.50 (1017)	1.72 ± 3.43 (175)
7-amino-flunitrazepam	0.12 ± 0.19 (715)	0.19 ± 0.29 (133)*
Methotrimeprazine (*Levomepromazine*)	0.57 ± 1.16 (181)	0.63 ± 1.22 (39)

*p = 0.000,356.

TABLE 3—*The 100 most commonly detected substances in the postmortem material, arranged according to the number of positive cases. Note that the table displays data for the years 1992–1994, whereas a number of cases from 1995 are included in Table 1.*

Substance	1992	1993	1994	Total
Acetaminophen (paracetamol)	478	493	543	1514
Propoxyphene (dextropropoxyphene)	300	345	374	1019
N-desmethyldiazepam (nordazepam)	253	253	297	803
Diazepam	234	246	285	765
7-amino-flunitrazepam	225	256	237	718
7-amino-nitrazepam	197	211	189	597
Carbamazepine	155	198	176	529
Dihydropropiomazine	159	168	181	508
Codeine	122	154	151	427
Morphine	128	137	161	426
Lidocaine	148	148	110	406
Propiomazine	117	113	122	352
Amitriptyline	95	95	83	273
Clomipramine	87	87	71	245
Nortriptyline	93	84	67	244
Amphetamine	88	64	82	234
Isopropanol	77	65	76	218
Norpropoxyphene	64	86	61	211
Alimemazine (trimeprazine)	78	56	71	205
Theophylline	70	75	57	202
Desmethylclomipramine	79	60	62	201
Methotrimeprazine (levomepromazine)	68	57	70	195
Citalopram	0	32	156	188
Oxazepam	58	55	56	169
Thioridazine	53	54	57	164
Tetrahydrocannabinol	52	53	58	163
Orphenadrine	55	55	45	155
Phenytion	40	48	52	140
Mianserin	53	44	36	133
Desmethylcitalopram	0	23	107	130
Desmethylmethotrimeprazine	49	40	37	126
6-acetylmorphine	38	47	40	125
Nitrazepam	64	33	25	122
Desmethylalimemazine	53	31	35	119
Quinine/Quinidine	38	33	36	107
Flunitrazepam	29	44	32	105
Verapamil	36	35	34	105
Ketamine	32	35	35	102
Carisoprodol	26	39	34	99
Phenazone	39	29	30	98
Thiopental	44	36	18	98
Phenobarbital	28	24	38	90
Promethazine	27	27	33	87
Norverapamil	32	26	24	82
Caffeine	5	28	47	80
Zopiclone	3	17	59	79
Trimipramine	26	26	20	72
Remoxipride	24	38	6	68
Meprobamate	22	28	17	67
Midazolam	17	25	25	67
Chlormezanone	24	27	15	66
Moclobemide	21	22	22	65
Chlordiazepoxide	26	17	21	64
Desmethylmianserin	31	22	11	64
Hydroxizine	20	26	16	62
Insulin	17	20	25	62
Oxomoclobemide	19	22	20	61
Mepivacaine	16	26	15	57
Paroxetine	12	24	20	56
Cyanide	22	22	10	54
Diltiazem	17	21	14	52
Desmethyltrimipramine	18	20	13	51
Maprotiline	14	19	17	50
Methanol	19	13	17	49
Alprazolam	13	21	14	48
Chlorzoxazone	19	11	17	47
Melperone	12	21	14	47
Desmethylpromethazine	22	11	12	45

TABLE 3—*Continued*

Substance	1992	1993	1994	Total
Pentobarbital	13	18	12	43
Oxyrazine	13	15	14	42
Fluvoxamine	18	9	15	42
Salicylate	8	12	22	42
Desipramine	14	18	8	40
Propranolol	9	15	16	40
Chloroquine	14	12	12	38
Lobemidone	15	12	10	37
Methadone	15	11	11	37
N,N,dimethyl-4,4-diphenyl-3-buthen-2-amine	13	15	6	34
Chlorpromazine	11	18	5	34
Dihydromelperone	8	18	8	34
Ephedrine	10	14	8	32
Trimethoprim	7	16	9	32
7-amino-clonazepam	5	6	19	30
Clozapine	9	7	12	28
Fluconazole	10	5	13	28
Pethidine	13	8	7	28
Trichloroethanol	7	10	11	28
Digoxin	8	7	11	26
Biperiden	9	8	7	24
Ethylmorphine	6	7	11	24
Chlorprothixene	8	8	4	20
Mesoclopramide	0	2	18	20
Cyclizine	1	5	13	19
Imipramine	6	10	2	18
Haloperidol	10	3	3	16
Diphenhydramine	8	3	4	15
Phenmetrazine	0	5	10	15
Clomethiazole	3	5	6	14
Naproxen	1	5	7	13
Trimidone	1	3	9	13

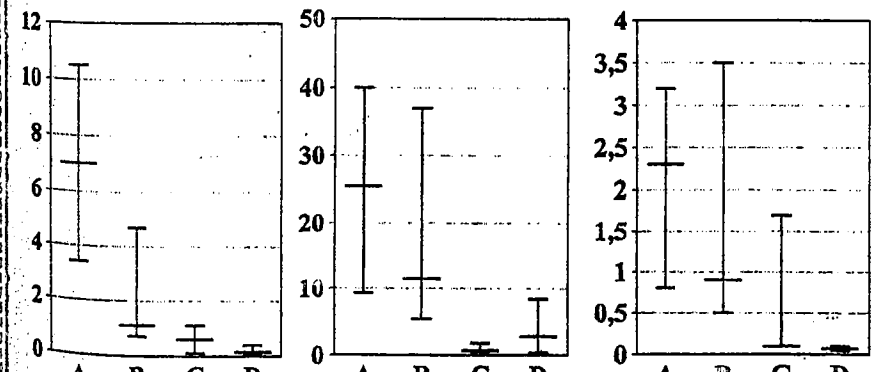


FIG. 1a–c—*Line plots showing the concentrations of citalopram (1a), carisoprodol (1b), and methotrimeprazine (1c), respectively. Group A = fatal intoxication with the substance exclusively. Group B = fatal intoxication with the substance in combination with other drugs and/or alcohol. Group C = other cause of death without incapacitation due to drugs. Group D = Concentrations in whole blood from suspected drugged drivers. In groups A–C concentrations refer to femoral blood. These line plots serve as examples of the variations of the overlap between intoxications and controls displayed by different drugs.*

Case 2:08-md-01968 Document 590-27 Filed 09/08/11 Page 64 of 64 PageID #: 24539